From THE DEPARTMENT OF CLINICAL SCIENCE AND EDUCATION, SÖDERSJUKHUSET

Karolinska Institutet, Stockholm, Sweden

DIAGNOSTICS AND TREATMENT OF NUT AND PEANUT ALLERGY

Josef Brandström

Karolinska Institutet

Stockholm 2018

Published by Karolinska Institutet.
Cover image by Martin Ask
Printed by Eprint AB 2018

ISBN 978-91-7831-007-4

Diagnostics and treatment of nut and peanut allergy

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Josef Brandström

Principal supervisor:
Docent Caroline Nilsson
Karolinska Institutet
Institutionen för klinisk forskning och utbildning, Södersjukhuset

Co-supervisor(s):
Docent Anna Nopp
Karolinska Institutet
Institutionen för klinisk forskning och utbildning, Södersjukhuset

Docent Gunnar Lilja
Karolinska Institutet
Institutionen för klinisk forskning och utbildning, Södersjukhuset

Med.dr. Mirja Vetander
Karolinska Institutet
Institutionen för miljömedicin

Opponent:
Med.dr. Anna Kaarina Kukkonen
Helsinki University
Skin and Allergy Hospital

Examination board:
Docent Guro Gafvelin
Karolinska Institutet
Institutionen för Medicin, Solna
Enheten för immunologi och allergi

Professor Göran Wennergren
Göteborgs Universitet
Institutionen för kliniska vetenskaper
Avdelningen för pediatrik

Docent Anders Åhlin
Karolinska Institutet
Institutionen för klinisk forskning och utbildning, Södersjukhuset

ABSTRACT

Background: The prevalence of allergy to foods has increased over the last decades and among children in Europe as many as 8 % have an allergy to one or more types of food. However, many children have received an incorrect diagnosis of food allergy due to shortcomings of available diagnostic tests, especially in the case of suspected allergy to nuts or peanuts. Newer diagnostic tools, like component-resolved diagnostics (CRD) and basophil activation test (BAT), e.g., basophil allergen threshold sensitivity (CD-sens), have shown an improved diagnostic accuracy compared with older tests.

The most severe acute manifestation of allergy, the anaphylactic allergic reaction, is most commonly caused by an allergy to peanut or nuts, and there have been no treatments available that might change the course of the disease. While disease-modifying allergen immunotherapy has for decades been offered as routine practice for the treatment of pollen or house dust mite allergy, severely food-allergic patients have had to settle for strict elimination diets and use of emergency medication in case of accidental intake. During the past decade, oral immunotherapy (OIT) has emerged as a potential disease-modifying treatment for food allergies, but OIT needs to be refined before it can become widely implemented. Major limitations of OIT have been frequent allergic reactions and that patients with a more severe allergy have a less favorable treatment outcome. The anti-IgE antibody omalizumab has been shown to increase the tolerated amount of food allergen among food-allergic patients (as long as the treatment continues) and facilitate initiation of immunotherapy in patients with severe allergies.

Objectives: Hazelnut study: To evaluate the new diagnostic tests CRD and CD-sens in children with a suspected hazelnut allergy. FASTX study (Food Allergen Suppression Therapy with Xolair®): To evaluate safety and efficacy of oral immunotherapy with adjuvant omalizumab in severely peanut-allergic patients.

Methods: In the study of diagnostic tests for hazelnut allergy, we used CRD to measure IgE antibody (ab) levels to the hazelnut components Cor a 1, Cor a 8, Cor a 9 and Cor a 14 in 40 children with a doctor's diagnosis of suspected hazelnut allergy. We also assessed basophil allergen threshold sensitivity (CD-sens) to hazelnut and CRD to hazelnut components and compared the concordance of these tests to double-blind placebo-controlled food challenge (DBPCFC). In the FASTX study, open-label omalizumab was given to 23 severely peanut-allergic adolescents, with the aim of increasing the amount of peanut they could safely ingest so that OIT could be safely initiated. Omalizumab doses were titrated until CD-sens analyses indicated a very low reactivity to peanut allergen stimulation. Thereafter, an open peanut challenge was performed, assessing the tolerated peanut dose while on omalizumab, and peanut OIT was started the following day. After reaching the maintenance dose of 10 g of peanuts, the protective omalizumab treatment was phased out with guidance from CD-sens and the clinical picture.

Results: DBPCFC revealed that only 8/40 of the patients with a suspected hazelnut allergy were allergic to hazelnuts. The diagnostic accuracy of the new diagnostic tests, CD-sens and IgE-ab to Cor a 9 and Cor 14, were far superior to the previously available tests (IgE-ab to hazelnut, Cor a 1 and Cor a 8). IgE-abs to Cor a 9 and Cor a 14 were present in all hazelnut-allergic patients; for Cor a 9 the median IgE-ab level was 4.5 kU_A/l (range 0.7–97.5) among hazelnut-allergic children, compared with 0.1 kU_A/l (range < 0.10–36.2) ($P < 0.01$) in the hazelnut-tolerant group. The levels of IgE-ab to Cor a

14 were 5.6 kU_A/l (0.9–78.7) for the hazelnut-allergic group and 0.04 kU_A/l (< 0.10–13.9) in the hazelnut-tolerant group ($P < 0.001$).

Median CD-sens among allergic patients was 8.9 compared with 0.05 in tolerant patients ($P = 0.05$). The diagnostic accuracy of CD-sens to hazelnut was maintained in subgroup-analyses where patients without IgE-ab to Cor a 9 or Cor a 14 > 0.35 kU_A/l were excluded from analyses.

After omalizumab treatment, all 23 patients passed a peanut challenge of > 3 g of peanuts (median 10 g) and were started on OIT the following day. Among the 14 patients who went through a peanut challenge prior to enrollment, the tolerated dose increased at least 50-fold (median). However, 15/23 patients needed an increased omalizumab dose in order to accomplish a suppression of CD-sens. All 23 patients successfully reached the 10 g maintenance dose. After a median of 23 months of OIT, 11/23 (48 %) of the study subjects had been able to discontinue omalizumab while continuing and tolerating OIT and thereafter passing an open peanut challenge. Systemic reactions (n = 43) occurred with a frequency of 0.3 % of OIT doses and adrenaline was administered after 0.1 % of the doses. We found that successfully treated patients had significantly lower baseline CD-sens and lower IgE-ab to peanut and peanut components Ara h 1, Ara h 2 and Ara h 3 compared with patients unable to discontinue the protective omalizumab treatment. OIT induced an increase of IgG4-ab to peanut, Ara h 2 and Ara h 6 that was significantly higher in successfully treated patients. A substantial proportion, 6/23 (26 %) of the patients dropped out of the study, mainly due to fear of allergic reactions and an abomination for the taste of peanuts.

Conclusions: CD-sens to hazelnut and component-resolved diagnostics can improve the accuracy when diagnosing hazelnut allergy in pediatric patients. CD-sens may further improve the diagnostic accuracy in cases when the diagnostic work-up using CRD has been inconclusive.

The anti-IgE-ab omalizumab can efficiently increase the tolerated peanut dose, which in turn allows for a safer practice of peanut oral immunotherapy in severely allergic patients.

Peanut oral immunotherapy induces an increased tolerance to peanuts; the increased tolerance is at least partially explained by the production of protective allergen-specific antibodies of IgG4-subtype.

Despite the increased tolerance, allergic reactions continuously occur during pOIT. We need to find ways to minimize this major limitation before OIT can be widely implemented; development of hypoallergenic OIT preparations, use of immune stimulatory adjuvants and improved patient selection might help in accomplishing a safer and more effective treatment.

LIST OF SCIENTIFIC PAPERS

I. Brandstrom J, Nopp A, Johansson SG, Lilja G, Sundqvist AC, Borres MP, Nilsson C, Basophil allergen threshold sensitivity and component-resolved diagnostics improve hazelnut allergy diagnosis. Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology 2015;45: 1412-8.

II. Brandstrom J, Vetander M, Lilja G, Johansson SG, Sundqvist AC, Kalm F, Nilsson C, Nopp A, Individually dosed omalizumab: an effective treatment for severe peanut allergy. Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology 2016.

III. Brandström J, Vetander M, Sundqvist A-C, Lilja G, Johansson S.G.O., Melén E, Sverremark Ekström E, Nopp A, Nilsson C. Individually dosed omalizumab enables peanut oral immunotherapy in severely peanut allergic adolescents.
Submitted

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LIST OF ABBREVIATIONS

Ab	Antibody
ABA	Allergen-binding activity
AE	Adverse event
AI	Allergen immunotherapy
aiTreg	Allergen induced T regulatory cell
Ara h	*Arachis hypogaea*
BAT	Basophil activation test
CD (as in CD 63)	Cluster of differentiation
CD-sens	Basophil allergen threshold sensitivity
Cor a	*Corylus avellana*
CRD	Component-resolved diagnostics
ELISA	Enzyme-linked immunosorbent assay
EPIT	Epicutaneous immunotherapy
ED50%	Allergen dose inducing 50 % of max CD63 % upregulation
FACS	Fluorescence-activated cell sorting=Flow cytometry
FASTX	Food Allergen Suppression Therapy with Xolair®
FcεRI	High-affinity IgE antibody receptor 1
FOXP3	Forkhead box protein 3
DBPCFC	Double-blind placebo-controlled food challenge
HR2	Histamine receptor 2
IL	Interleukin
mAb	Monoclonal antibody
OFC	Oral food challenge
OIT	Oral immunotherapy
pOIT	Peanut oral immunotherapy
ROC	Receiver operating characteristics
SCIT	Subcutaneous Immunotherapy
SPT	Skin prick test
Th	Helper T-lymphocytes (T helper cells)
Treg	Regulatory T-lymphocytes (T regulatory cells)

1 INTRODUCTION

Food allergy is a major health concern, affecting as many as 8 % of children.[1,2] The prevalence of allergy to peanuts or nuts (and food allergy in general) seems to be increasing[3-5] and unlike allergies to cow's milk[6] or hen's egg,[7] peanut and nut allergies are seldom outgrown.[8,9] Peanuts and nuts are the most common triggers for anaphylaxis among children.[10]

Food allergies are most often IgE-mediated. An allergic reaction might take place when an IgE-sensitized individual[11] (someone who has IgE antibodies capable of binding one or several allergens) encounters an allergen[12] (usually a protein) that the IgE antibodies can recognize and bind to. However, far from all sensitized individuals experience symptoms upon allergen exposure.[13-15] This can partially be explained by the fact that the ability to induce an allergic reaction differs from one allergen to another. Within an allergen source, such as the hazelnut, there are several allergens[16] of which some, like the highly stable seed storage protein Cor a 14, are known to be highly capable of inducing allergic symptoms,[17] in contrast to the birch pollen-related hazelnut protein Cor a 1.[15,18] Traditional diagnostic tests, the skin prick test and analysis of IgE-ab to an allergen source, cannot discriminate between test results which are positive due to sensitization to a highly potent allergen like Cor a 14 and those positive due to sensitization to Cor a 1. Over the years, many patients have received an inaccurate diagnosis of being allergic.

New diagnostic tests, like component-resolved diagnostics (CRD) and basophil activation test (BAT), have improved the diagnostic accuracy. In CRD, IgE-sensitization to individual allergens is detected, as opposed to the traditional tests, which detect presence of IgE antibodies against any of the proteins (both those of high and low clinical importance) within the allergen source.[19] In BAT, basophilic granulocytes, one of the two most important effector cell types for IgE-mediated allergy (mast cells being the other), are stimulated with allergen extracts in vitro. Basophils that react with degranulation upon allergen stimulation can be detected through flow cytometry. Basophil allergen threshold sensitivity, CD-sens,[20] is a BAT method where the cells are stimulated with an allergen at several concentrations. CD-sens discriminates peanut-allergic from peanut-sensitized but tolerant patients with high accuracy,[21,22] and can be used to monitor treatment effect in allergen immunotherapy.[23,24]

Subcutaneous allergen immunotherapy (SCIT) has been used as a treatment for pollen allergy for over a century[25] and more recently for other allergies, such as insect venom allergy.[26,27] It is long since established as efficacious in causing symptom relief (e.g., in pollen allergy) and preventing anaphylaxis (in venom allergy).[26,28,29] However, there have not been any approved disease-modifying treatments for patients suffering from food allergies; SCIT has been tried for peanut allergy, but severe adverse events (AEs) occurred frequently.[30,31] In the last 15 years, oral immunotherapy (OIT) has emerged as a potential therapy for food allergies.[32,33] Short-term outcomes of OIT are generally good.[33,34] The downsides of OIT are that most subjects experience adverse events,[35] that protection seems to vanish if OIT is stopped, even after years of treatment[35] and it seems that OIT is less effective in patients with severe allergies and high IgE-ab levels.[34-36]

An anti-IgE antibody, omalizumab, approved for the treatment of allergic asthma, has been shown to increase the tolerated dose of peanuts among peanut allergic subjects.[37,38] When combined with OIT, omalizumab has been shown to facilitate OIT by reducing AEs and allowing for faster up-dosing of OIT.[36,39,40]

This thesis aimed to evaluate the newer diagnostic tests CRD and CD-sens in patients with a suspected hazelnut allergy. We also aimed to provide and evaluate omalizumab-facilitated peanut oral immunotherapy among severely peanut-allergic adolescents.

2 BACKGROUND

2.1 FOOD ALLERGY

Food allergy is a common disorder; exactly how common depends on geographic location, how the disorder is defined and how it is measured. It is estimated that up to 8 % of children in Europe and North America are allergic to some kind of food.[1,2]

2.1.1 Definition

Food allergy can be defined as "an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food."[41] It could be argued that this definition is not 100 % accurate, e.g., when it comes to reproducibility.

In contrast, food intolerance has a non-immune-mediated pathophysiology. Symptoms in such disorders could be due to metabolic causes, such as lack of enzymes in lactose intolerance[42,43] or glucose-6-phosphatase dehydrogenase deficiency (favism),[44] pharmacological causes, as in sensitivity to biogenic amines,[45] or unknown causes.

2.1.2 Classification of food allergy

Food allergy is commonly classified into IgE-mediated and non-IgE-mediated food allergy, but can be caused by both immunologic mechanisms at once. IgE-mediated food allergies are far more common (especially in the case of nut or peanut allergy) and will be discussed in detail in this thesis.

The non-IgE-mediated food allergies include diseases such as celiac disease,[43] FPIES[46] (food protein-induced enteropathy syndrome), Heiner's syndrome,[47] eosinophilic esophagitis and eczema, where T-cells have a major role in the development of symptoms. However, for eczema, IgE is sometimes involved in the pathophysiological process,[48] and IgE-abs might also be involved in the pathophysiology of eosinophilic esophagitis and Heiner's syndrome, at least for a subset of patients.

2.1.3 Epidemiology

Exact and consistent prevalence figures for food allergy are hard to find, as they vary by geographic location, age, and diagnostic criteria.[1] A 14.5 % point prevalence of self-reported food allergy has been reported for northern Europe as compared with 3.5 % for southern Europe, while only 1.6 % and 1.8 % respectively had a positive skin prick test result to the corresponding food.[49] In a population-based cohort of children aged 7–8 years from northern Sweden, the parent-reported prevalence of food allergy and food hypersensitivity was 21 %, Strinnholm et al. 2006.[50] In the same cohort, the prevalence of self-reported allergy to milk, egg, wheat or cod at age 12 years was 4.8 %. About 1/3 (1.4 % of total) received a doctor's diagnosis of food allergy after clinical evaluation (including laboratory work-up) and in 1/6 (0.6 % of total) the allergy was confirmed by the gold standard double-blind placebo-controlled food challenge.[51]

The prevalence of food allergy has increased over the last decades and recent reports suggest that the prevalence might be as high as 8 % among children.[1-3,52-54] In the pediatric population, the most common food allergies are to milk or egg, followed by allergy to wheat, peanuts and or tree nuts.[55,56]

Peanut allergy is the most common "nut" allergy. As stated above, exactly how common it is depends on the population studied and how it is defined, but the average pediatric prevalence seems to be

around 2 % in Western countries.[13,57] Prevalence might be higher among Swedish children; in the Stockholm-based BAMSE cohort,[58] as many as 5.9 % of the 8-year-olds had both self-reported peanut allergy and IgE-sensitization, although cross-reactivity from birch pollen allergy probably explains both symptoms (oral allergy syndrome) and sensitization in some individuals. Epidemiologic data on tree nut allergy is inconsistent; in a recent systematic review[59] of 36 studies the "probable tree nut allergy prevalence" ranged from 0.05 % to 4.9 % (combination of one or more of the following: history, laboratory tests and doctor's diagnosis). The most common tree nut allergy in Europe is hazelnut allergy, whereas allergies to walnut or cashew are more common in the US.

When it comes to the most severe type of allergic reaction, anaphylaxis, Vetander et al. estimated that peanuts cause 19 % of food-induced anaphylaxis among Swedish children. Tree nuts as a group also caused 19 % of anaphylaxis (cashew nut 8 %, hazelnuts 2 %). In comparison, egg or milk was the eliciting food in 12 % and 6 % of cases, respectively, and in 23 % of cases, the eliciting food remained uncertain.[10]

2.1.4 Pathophysiology

The immune system is a very complex and tightly controlled system. It protects us against a wide range of harmful and potentially lethal threats such as bacteria, viruses, and malignantly transformed cells, by attacking and killing them.[60] At the same time, a well-regulated immune system stays tolerant of its host (in other cases, auto-immune diseases may develop) and of non-harmful substances, such as foods.[61] Allergies are a consequence of a dysregulated or maladaptive immune system that reacts in an undesired manner upon exposure to allergens.

An imbalance between cytokines produced by T-helper (Th) cells type 1 and 2, with an excess of Th2 cell cytokines, has been one of the main explanatory models of the development of food allergy. The shift to Th2-dominance leads to an increased production of IgE-type abs, as well as pro-allergenic cytokines such as IL-4, IL-5, and IL-13.[62] The reason for this imbalance is thought to be multifactorial. Environmental factors such as exposure to cigarette smoke,[63,64] pollutants,[65] microbial exposure,[66] timing of introduction of solid foods,[67,68] allergen exposure, including through which route exposure occurs: oral vs. dermal,[69,70], lifestyle,[71] and lastly, but very importantly, hereditary factors and other atopic diseases, such as eczema (the atopic march), all affect the risk of becoming food-allergic.[72-74] The Th1-Th2 imbalance, or this imbalance in combination with the above mentioned factors, is thought to induce B-lymphocytes to produce IgE-abs instead of other classes of Ig-abs. When an individual has developed IgE-abs directed against an allergen, he/she has become sensitized.[11]

2.1.5 The IgE-mediated allergic reaction

The IgE produced by a sensitized individual can be found both as free IgE-abs in the circulation and as IgE-abs bound to target cells like basophilic granulocytes (in blood) or mast cells (in tissues). When the sensitized individual's immune system encounters the allergen, an IgE-mediated reaction might take place. In order to activate the mast cells or basophils, the allergen has to cross-link IgE-abs, bound to high affinity FcεRI receptors.[75] If enough crosslinks (hundreds to thousands) have been established, an intracellular signal is produced, strong enough to activate the cells.[76] The activation leads to a series of events eventually leading to degranulation, which in turn leads to the release of histamine, tryptase, carboxypeptidase, and other mediators that cause allergic symptoms such as erythema, hives, bronchoconstriction, and rhinitis.[11]

2.1.6 Allergens and cross-reactivity

Allergic reactions are elicited by allergens. Accordingly, allergens are molecules/substances/antigens that may induce allergic reactions and to do so, the allergens have to be able to bind and cross-link IgE-abs (for IgE-mediated allergies).[77] Most allergens are also capable of inducing production of IgE-abs specific for the antigen, i.e., they have the ability to sensitize.[11] Allergens are almost exclusively proteins, and although many proteins are capable of inducing an allergic response, some are far more likely to cause allergies. These common allergens share features such as: many epitopes (binding sites) for IgE-abs, similar molecular weights, and resistance to degrading enzymes and heat.[78,79]

Cross-reactivity can occur, as allergens from different sources can be so homologous that IgE-abs produced in response to exposure to one allergen can bind to other similar allergens from other sources. An example of high relevance is hazelnut and birch pollen cross-reactivity: The main allergen of birch pollen, Bet v 1,[80] is a PR-10 protein (pathogenesis related protein 10) that is homologous to and has similar epitopes as the Cor a 1 protein (also a PR-10) in hazelnuts. Hence, an IgE-ab targeted for birch pollen also has the potential to bind (cross-react) to this hazelnut protein.[81] These cross-reactive IgE-abs are not as capable of inducing allergic reactions, since their capacity to bind and cross-link IgE-receptors on basophils and mast cells is not as strong as IgE-abs from primary sensitization. Also, Cor a 1 is unstable. Thus, digestion by saliva and gastric fluid and/or cooking degrades Cor a 1 which alters the epitopes' micro-structures so that they are no longer able to attract Cor a 1 IgE-abs.[82] This explains why a person with birch allergy who gets oral pruritus when eating raw hazelnuts can usually eat processed hazelnut without experiencing problems with oral symptoms.

2.2 NUT AND PEANUT ALLERGY DIAGNOSTICS

A thorough patient history is imperative in the diagnostic work-up of suspected food allergy. There are today several ways to diagnose food allergy, with a trade-off between costs and quality (Figure 1). To confirm a diagnosis of IgE-mediated allergy, or when patient history is not enough, an allergy test such as the skin prick test (SPT) or measurement of circulating IgE-abs in the blood is usually the first diagnostic step. Such tests can easily identify IgE-sensitized individuals, but unfortunately they are not as good at diagnosing food allergy adequately.[18,41] The discrepancy between sensitization (measured with SPT or specific IgE-abs) and allergic symptoms is aggravated when diagnosing allergy where cross-reactivity is common, e.g., diagnostics of nut allergies in the Nordic countries where birch pollen allergy is common.[83] The gold standard for diagnosing food allergy, the double-blind placebo-controlled food challenge, is expensive and associated with risks.[41] Therefore, improved diagnostics tools like component-resolved diagnostics and basophil activation tests are much needed.

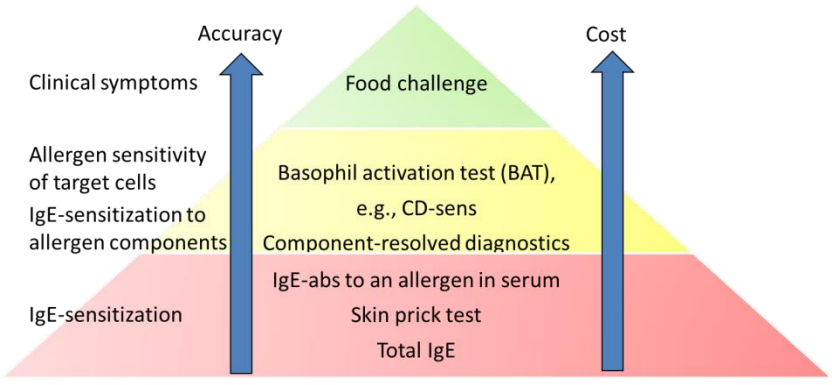

Figure 1. Inside pyramid: food allergy diagnostic tests. To the left: what these tests measure.

2.2.1 Skin prick test

SPT is a rapid and safe allergy test that allows for testing of one or more allergens simultaneously. The allergen is usually in the form of an extract. A drop of this solution is placed on the patient's skin (usually on volar side of the forearm) and the skin is thereafter superficially punctured with a small needle. In the presence of allergen-specific IgE-abs bound to mast cells in the skin the allergen might cause a local reaction of a wheal and flare (usually measured after 15 minutes) that is compared with negative (saline) and positive (histamine) controls.[84] A positive test is evidence of the patient being sensitized to the allergen, but the correlation to clinical allergy varies from one allergen to another and also with the size of the wheal.[85] Wheal sizes might also differ depending on who is performing the test[86] and on the extract being used.[18] False negatives do occur,[18] but in general the major drawback of SPTs is the low specificity (false positives).[84,87,88]

2.2.2 Allergen-specific IgE-abs

The presence of allergen-specific IgE-abs (or cross-reacting IgE-abs) is a prerequisite for the IgE-mediated allergic reaction to take place. IgE-abs are today easily measured in vitro, as commercial tests are available for many allergens. The tests use the ELISA method (enzyme-linked immunosorbent assay) to detect IgE-abs capable of binding the allergen of interest. Briefly, the allergen is bound to a solid phase and the patient's serum (or blood) is added. After incubation, when IgE-abs can bind to the allergen, the serum is washed away, leaving only allergen-bound IgE-abs. Anti-IgE molecules with fluorescent markers are then added, the sample is washed again, and thereafter the amount of allergen-bound IgE-abs is measured through spectrophotometry.[89] Like in the skin prick test, a positive test is not equivalent to allergy. This is partly due to the fact that an allergenic food often consists of many proteins, which in theory can serve as allergens. Both measurement of IgE-abs and SPT demonstrate presence of IgE-abs to any of these proteins within the allergen; however, some of these proteins are unstable and/or have lower ab-affinity due to secondary sensitizations as in the case of cross-reactivity to, e.g., birch pollen.[13,83] Also, monovalent allergens are capable of binding one IgE molecule each, which may result in a positive test result, even though monovalent allergens cannot achieve the cross-binding of several IgE-abs required to activate basophils and mast cells.

2.2.3 Component-resolved diagnostics (CRD)

By separating, purifying, and identifying the different allergens within an allergen source it is possible to analyze IgE-abs to individual proteins within an allergen source instead of analyzing IgE-ab levels to whole allergens. These different allergens/proteins are the "components" in "component-resolved diagnostics," a term coined by Valenta et al. in 1999.[19] IgE-sensitization to the components is measured using the same methodology as in allergen-specific IgE-ab testing.

The components' names derive from their Latin names (genus and family). Taking hazelnut as an example: hazelnut has the Latin name *Corylus avellana*: the hazelnut proteins are named "Cor a" followed by a number, e.g., Cor a 1 and Cor a 14, while examples of peanut (*Arachis hypogaea*) components are Ara h 2 and Ara h 8.

By looking at sensitization patterns to different allergens within an allergen source, diagnostic accuracy can be improved. There is now rapidly increasing knowledge of which IgE-sensitization

patterns to certain proteins that are associated with allergy and, vice versa, which sensitization patterns that are associated with cross-reactivity to e.g. pollen.

Patients sensitized to PR-10 proteins only (Cor a 1, Ara h 8) have a high likelihood of being tolerant to hazelnut and peanut, respectively.[83,90] In nut and peanut allergy, it has been established that sensitization to proteins from the 2S albumin, 7S albumin and 11S globulin protein families are highly associated with clinical allergy.[17,91] These seed storage proteins are highly resistant to heat and enzymatic degradation and hence remain largely intact when they come in contact with the patients' immune system. They do not cross-react with major aeroallergens such as birch or timothy, thus making a false-positive test result less likely. Taking peanut as an example, sensitization to the 2S albumin protein Ara h 2 has in several studies been shown to correlate excellently with clinical allergy to peanut.[21,92] By combining this information on component (protein) properties with the patient's sensitization pattern to these individual components, diagnostic accuracy can be improved.[15,92] The increased diagnostic accuracy achieved using component-resolved diagnostics has proven more beneficial when diagnosing food allergies from the plant kingdom than for animal products,[93] e.g., peanut[13,21,91,94,95] and nut allergy, hazelnut,[15,17,90,96] cashew,[97-100] walnut,[101] and soy.[102,103] Still, component-resolved diagnostics is far from perfect and even with moderately elevated IgE-ab titers to storage proteins, such as Ara h 2 in peanut or Cor a 9 and 14 in hazelnut, we still find patients tolerant to the tested food.[104] Thus testing and interpreting test results should always be carried out in the light of patient history.

2.2.4 Oral food challenge (OFC)

The double-blind placebo-controlled food challenge is the gold standard for diagnosing food allergy.[41] OFCs should be carried out by experienced staff in a hospital setting, since clinical evaluation of symptoms at OFCs is often difficult and severe reactions requiring emergency room treatment do occur.[105] Usually, the food is given in very small amounts (milligrams) and stepwise escalated every 30 minutes until doses of several grams are reached. Rarely, in case of certainty of tolerance, single dose OFC of several grams of the food can be given right away. When diagnosing food allergies, and in research, the double-blind placebo-controlled food challenge (DBPCFC) is preferred in order to minimize the impact of psychological effects.[106] However, since health care resources are often limited, open food challenges are more common.

2.3 BASOPHIL ACTIVATION TEST

Basophils and mast cells initiate the IgE-mediated allergic reaction, thus making them interesting targets for allergy diagnostic tests. While mast cells are mainly localized in tissue and thereby hard to harvest for in vitro analysis, basophils are readily available in blood.[11,107]

When IgE-abs bound to high affinity FcεRI receptors on mast cells/basophils are stimulated with antigens in large enough quantities, the basophils react by secreting mediators such as histamine, tryptase, carboxypeptidase and IL-4, which are released into the tissue or blood stream.[108] While tryptase, for instance, is known to be released from basophils and mast cells in severe allergic reactions, the tryptase levels correlate poorly with the severity of the allergic reaction, especially in children.[90] There are several ways of effectively measuring basophil activity to an allergen which all go under the name basophil activation test (BAT) or similar, but they vary when it comes to methodology. Basophils can either be stimulated with different dilutions of the allergen of interest, rendering the basophils' ''allergen sensitivity'' as in the CD-sens method,[109] or they can be stimulated

at a single concentration, revealing the reactivity, i.e., how strongly these basophils react at a given concentration.[110,111]

BAT as a method utilizes flow cytometry to detect basophils, and CD203c is commonly used as a marker for identifying basophils.[112] When basophils are activated, granules containing, e.g., histamine are released from the cell. The same granules also contain CD63; histamine release and CD63 cell surface exposure occur simultaneously.[113] In contrast to histamine, CD63 remains on the cell surface and by using flow cytometry, activated CD63-positive basophils can be identified. The ratio of CD203c/CD63 double positive cells (identified using flow cytometry) to CD203c single positive cells gives us the percentage of basophils that are activated by the stimulation.

2.3.1 CD-sens

Basophil allergen threshold sensitivity, CD-sens, is a BAT method where basophils from peripheral blood are stimulated with several (usually 8–10) allergen concentrations. Since it is a test performed on whole blood, serum components like antibodies and cytokines will be present, which might influence the basophils' sensitivity, just as they would in vivo. Like an oral food challenge, the CD-sens method will not only provide a dichotomous positive/negative result, but also provide information on how sensitive the basophils are to the allergen. The CD-sens value is calculated as follows: the eliciting allergen dose that induces 50 % (ED50 %) of maximum CD63 % upregulation of the dose-response curve is identified. ED50 % is then inverted and multiplied by 100. Hence, the higher CD-sens value, the more sensitive the basophils are to this particular allergen (Figure 2).[109]

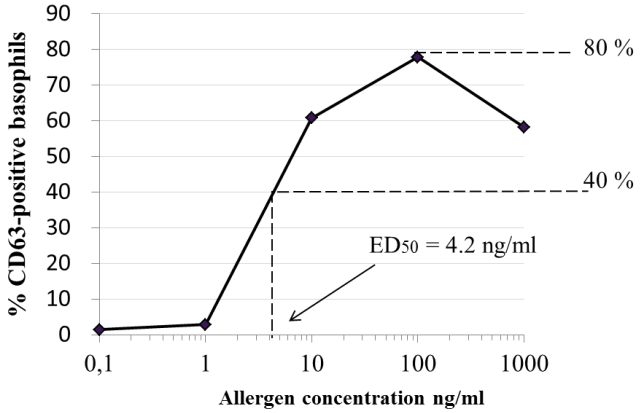

Figure 2. The allergen concentration that elicits a 50 % of maximum CD63 % upregulation (ED50 %) is calculated from the dose-response curve. CD-sens= the inverted ED50 % x 100. Adapted (with permission) from Nopp A.[109]

2.3.2 Allergen-binding activity (ABA)

CD-sens is usually performed on whole blood samples, but it can also be performed in a sample where the plasma has been washed away, leaving only blood cells in the sample. When the plasma-depleted washed CD-sens value is higher than the whole blood CD-sens, there is an indication of the presence of factors that interfere with the allergic reaction. The magnitude of this blocking effect can be demonstrated as the ratio between the washed CD-sens and the whole blood CD-sens, named ABA (allergen-binding activity) by Johansson, Nopp et al.[109] ABA is induced in successful allergen immunotherapy[35,114] (AI), hence ABA can be used as a marker when assessing the efficacy of AI.[23,24]

2.3.3 Clinical implications for CD-sens

Many cases of food allergy or suspected food allergy are not hard to diagnose correctly for a trained allergist with the help of SPT and/or IgE-ab measurements, especially as we are now routinely using CRD (component-resolved diagnostics). But in a subset of patients there might still be doubts as to whether a positive sensitization test is due to cross-reactivity or is indicative of a more severe allergy (even after CRD). A food challenge would then be the most appropriate diagnostic investigation, but might not be feasible. In the diagnostic work-up of idiopathic anaphylaxis in poly-sensitized individuals numerous food challenges might be needed. In situations like this, CD-sens could be a useful diagnostic test. Basophil allergen threshold sensitivity correlates very well with the outcome of DBPCFC in patients with a suspected peanut allergy.[21] In addition to distinguishing between tolerant and allergic patients, the CD-sens method might be able to grade the severity of peanut allergy,[115] although confirming studies are needed. CD-sens can be analyzed for virtually any allergen, provided that suitable allergen extracts are available. CD-sens can also be used in the diagnostic work-up of patients with allergic asthma and allergic rhinitis, as it has been shown to correlate significantly with both nasal and bronchial allergen challenges.[116]

As described, CD-sens is useful when monitoring immunotherapy. In allergen immunotherapy IgE-ab levels tend to increase initially, making tests based on IgE-ab detection unsuitable for following treatment effect.[117] In anti-IgE treatment with the monoclonal antibody omalizumab, traditional IgE measurements cannot be used. IgE-ab levels in serum increase during omalizumab treatment since omalizumab binds free IgE-abs resulting in IgE-omalizumab complexes with a longer half-life compared with free-IgE.[118] In omalizumab-treated patients, suppressed basophil allergen sensitivity correlates with a highly increased clinical tolerance.[23,109]

2.3.4 Summary of nut and peanut allergy diagnostics

The need for diagnostic methods varies from case to case; sometimes there is no need of any tests or a skin prick test or serum IgE-ab test is sufficient, while other cases call for new and more advanced tests or food challenges. Figure 3 illustrates key factors in IgE-mediated allergic reactions. It also illustrates where the previously mentioned allergy diagnostic tests are used.

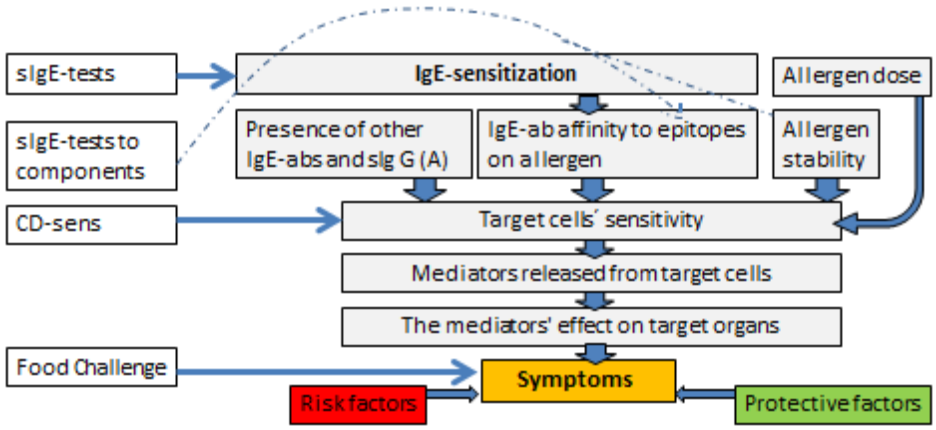

Figure 3. IgE-sensitized individuals do not necessarily experience symptoms upon contact with the allergen. Several factors, like allergen dose, grade of sensitization, presence of blocking antibodies, overall state of the individual at that time etc. will all have an impact on the clinical outcome after allergen exposure. The left column lists diagnostic tests, arrows indicate where, in this chain reaction, the effect that the test is measuring is found. The further down in the figure the test is found the better - at least in theory. Dotted lines indicate indirect assessment of allergen stability and affinity (based on experience at a population level or a pre-clinical level). ***Skin prick test is not included in this figure; in theory it measures a mediator effect on the target organ, but in reality it is about as accurate as IgE-ab analyses.***

2.4 TREATMENT OF FOOD ALLERGY

Until recently and before the studies within this thesis were planned and carried out, there was no approved disease-modifying treatment for food allergy. However, the European Academy of Allergy and Clinical Immunology (EAACI) recently published guidelines stating that allergen immunotherapy might be considered for carefully selected patients at specialized centers.[119] Prior to these guidelines (and even now, for the majority of patients), avoidance, emergency medication in case of accidental exposure, and patient education are recommended. In case of mild food allergies, this might be enough. But for many patients, their allergy is a considerable disability. Always facing the risk of having severe allergic reactions leads to significant negative effects on quality of life for the patients and their relatives. Few are aware that children with severe food allergy rank their quality of life as worse than children with diabetes.[120-122] Considering the fact that nut/peanut allergy is rarely outgrown,[8,9] it is obvious that there is a need for new treatment options.

2.4.1 Allergen immunotherapy

Allergen immunotherapy (AI) is a treatment of a specific allergy that is carried out by presenting one (or a few) specific allergen(s) to the patient's immune system in gradually increasing doses. AI is generally thought to be allergen-specific and hence does not affect allergies other than the one for which the subject receives treatment. However, Thyagarajan et al. showed that egg- and peanut-allergic children treated with peanut OIT had significantly lower basophil activation upon stimulation with an egg extract after OIT than before OIT was started.[123]

2.4.2 Sub-cutaneous immunotherapy

The most widespread and studied form of AI is subcutaneous immunotherapy (SCIT). SCIT has been used as a treatment for pollen allergy for more than a century[25] and is now also used for insect venom allergies and perennial allergies against, e.g., cat and house dust mite; it is efficacious, safe and cost-beneficial.[28,29,124,125] However, in trials of SCIT for food allergy (peanut), frequent severe adverse events were observed, including one fatality.[30,31] Several routes of administration have been tried for food allergy AI: sub-lingual (SLIT), epicutaneous (EPIT), and oral (OIT), all described below, and rectal,[126] not further discussed.

2.4.3 Oral immunotherapy

Oral immunotherapy (OIT) is a type of AI in which the allergen is ingested. The basic concept is to give the allergen in gradually increasing doses as the patient's tolerance increases. Although experimental use of OIT has increased explosively over the last decade, it is not an entirely new concept. The first successful case report of treatment of an egg-allergic boy dates back over a century[127] and, in a case series from the 80s where 19 patients were treated mainly for egg or milk allergy, treatment success was reported in 14/15 cases adhering to the treatment protocol.[128]

In 2009, Jones et al. reported results from their OIT study of peanut-allergic children[33] (age 1–10 years), starting with 1 mg of peanut protein and then increased (if tolerated) to a maximum of 300 mg. A peanut challenge was performed after 4–22 months. At this challenge, 27/29 patients ingested 1.8 g peanut protein (cumulative dose 3.9 g) resulting in a treatment success rate of 93 % per protocol or 69 % by intention to treat. However, many of these patients, 38 %, had objective symptoms and were treated with antihistamines. Blumchen et al. found that 14/23 children (5–14 years) with a predominantly severe allergy to peanuts were able to reach a maintenance dose of at least 125 mg of

peanut protein (range 125–500 mg). OIT was then stopped for two weeks and followed by a DBPCFC to peanut where the subjects tolerated a median dose of 250 mg.[129] Making a fair comparison of these two studies, and the studies that followed, is not easy since study populations differ in terms of age and allergy severity, and the definitions of successful treatment also differ. In the Jones study, the patients ingested higher doses of peanut, but not all patients tolerated these doses. In the Blumchen study, OIT was withdrawn for two weeks prior to food challenge, which is known to be associated with fewer patients being tolerant to the food in the following food challenge.[35,36,130]

In the first published placebo-controlled study of peanut OIT, 16/19 patients reached the maintenance dose of 4 g peanut protein. In this study, the initial doses were low, perhaps explaining that fewer patients dropped out. At the following DBPCFC, all 16 patients ingested a cumulative dose of 5 g of peanut protein (~20 peanuts), with only one of them needing treatment with antihistamine. The placebo group ingested a median dose of 280 mg of peanut protein, 3/9 patients were treated with epinephrine.[131] The patients in the Jones study[33] continued with OIT after the DBPCFC (follow-up study[35]) until their peanut-specific IgE dropped < 2 kU/l. Then, OIT was stopped for one month, followed by a DBPCFC at which 5/5 patients passed a 5 g peanut protein challenge. The protocol was then changed: symptom-free patients with peanut IgE of < 15 kU/l underwent the same challenge and 7/8 passed. Finally, all patients who had been on OIT for five years (but had peanut IgE > 15 kU/l) went through a DBPCFC and, interestingly, only 1/12 (8 %) passed after being off OIT for 1 month. Still, the cumulative dose among patients not passing this food challenge was quite high, > 3 g on average.[35] This study also provided interesting information on baseline data associated with successful outcomes; the strongest predictor was low levels of peanut IgE-abs/total IgE ratio and peanut IgE-abs. Low Ara h 2 IgE-ab levels just before the final peanut challenge were associated with a favorable outcome; similar predictive laboratory data were also observed in a milk OIT study by Wood et al.[36]

The largest peanut OIT study to date is the STOP II study by Anagnostou et al.[34] This was an open placebo-controlled study of British children (1–16 years) with a peanut allergy of any severity. Treatment success was 49 %, measured as passing a 1.4 g peanut protein challenge after six months of OIT. In the placebo group, no patients passed the food challenge.

A double-blind study comparing peanut OIT/SLIT was carried out by Narisety et al.[132] They treated 21 peanut-allergic children (7–13 years) with active OIT plus placebo-SLIT or placebo-OIT plus active SLIT. After six months, threshold doses had increased, in median, from 21 mg to 496 mg in the active SLIT group and from 21 to7,246 mg for active OIT. Thus OIT-treated patients tolerated more than ten times higher doses than the SLIT-treated children. After 12 months, patients tolerating less than 5 g continued with a combination of SLIT and OIT, while patients tolerating 5–9 g continued with monotherapy (only OIT cases) for six months. This study also evaluated sustained unresponsiveness in the ten patients who passed a final food challenge of a cumulative dose of 10 g peanut protein. Here, a new challenge was performed after four weeks off therapy, where only 4/10 passed. In contrast, SLIT appeared to be safer and better tolerated and 9/10 patients in this group stayed in the study during the whole blinded phase, compared with 7/11 in the OIT group.

2.4.4 Sub-lingual immunotherapy

In SLIT, small doses of the allergen are placed under the tongue where it is presented to the local immune system. It seems that SLIT is not as effective as OIT,[132] the main reason probably being that only a limited dose can be administered. On the other hand, it seems to be better tolerated than OIT,[132,133] probably due to lower doses and administration in a tolerant body part (oral cavity), where

basophils and mast cells are not as common.[61] In a double-blind placebo-controlled study by Fleischer et al.,[134] 40 peanut-allergic patients (12–37 years old) were given either peanut SLIT or placebo SLIT for ten months, followed by a peanut challenge. A total of 70 % in the active group increased their highest tolerated dose at least ten-fold, compared with baseline; the median increased from 3.5 mg to 496 mg. In the placebo group, 15 % had a ten-fold increased threshold dose. Mostly mild allergic symptoms were reported for about 37 % of doses given, non-oropharyngeal symptoms were reported for about 7 % of administered doses. After this, the placebo patients crossed over to the active group. The whole study lasted three years, but 50 % dropped out. After three years, four patients were fully desensitized to 10 g of peanut. They were taken off therapy for eight weeks and then re-challenged. All of them, 10 % of the initial study population, passed this re-challenge and had thus achieved sustained unresponsiveness (SU).[133]

2.4.5 Epicutaneous immunotherapy

In EPIT, allergen presentation happens through the skin by usage of allergen-containing skin patches. In 2010, a blind placebo-controlled study showed that EPIT is a well-tolerated treatment for cow's milk allergy with tendencies towards increased tolerance (not significant).[135] In a study of peanut EPIT with patches containing 50, 100, and 250 µg of peanut protein or placebo there was a significant difference between the 250 µg and placebo patch in achieving a ten-fold increase in tolerated peanut dose in a sub-group of children aged 6–11 years, while there was no significant difference among adolescents/adults.[136]

2.4.6 Omalizumab

Omalizumab is a drug developed and approved for treatment of severe allergic asthma and chronic spontaneous urticaria. Its active substance is humanized monoclonal anti-IgE-abs of IgG-type. Omalizumab is administered as sub-cutaneous injections every second to fourth week and the dosage is based on total IgE-level and body weight.[137] Omalizumab only binds free circulating IgE and binds the Fc-part of the IgE-molecule, the same part of IgE-abs that binds to the FcεRI on target cells.[138] As a result, IgE bound to omalizumab cannot bind to the FcεRI receptors on mast cells or basophilic granulocytes. The declining amount of free IgE leads to a downregulation of FcεRI receptors and limits IgE-mediated allergic processes.[139] It is important to remember that omalizumab binds any IgE molecule, irrespective of if it is a specific IgE-ab directed at the allergen of interest or not. When levels of total IgE-abs are high, the drug cannot bind enough IgE-abs to make treatment effective; thus, omalizumab is only recommended in patients with a total IgE of 30–1,500 kU/l.[140] In patients with low IgE-ab levels the treatment is not approved, but if there is a clinically proven IgE-mediated allergy, treatment might still be effective if higher doses than recommended by the manufacturer are given (although still low doses).[141]

When used together with immunotherapy, omalizumab combined with SCIT for pollen allergy was superior to placebo plus SCIT, both in terms of severity of side effects and in terms of outcome.[142] In some cases, it is not even possible to start SCIT due to severe allergic reactions even at the lowest SCIT doses; here, omalizumab might facilitate the initiation of therapy.[143]

2.4.7 Omalizumab and food allergy

The mechanisms by which omalizumab exerts its effect indicate that it should have an effect on IgE-mediated diseases other than asthma. The positive effect on food allergies was observed by Rafi and colleagues, who followed 22 patients with both allergic asthma and food allergies: these patients

reported fewer events of food-induced symptoms (asthma, skin symptoms and anaphylaxis).[144] Before omalizumab was approved, Leung et al. studied a similar anti-IgE drug, TNX 901, and showed that it induced a dose-dependent increased tolerance to peanuts with the highest effect at the highest drug dose (450 mg).[138] Used as pre-treatment before initiation of OIT for food allergies, omalizumab has been shown to effectively increase the tolerated dose of the food.[23,39,40]

2.4.8 OIT with adjuvant omalizumab

In a pilot study on 11 milk-allergic children by Nadeau et al, from 2011,[40] omalizumab was given for nine weeks followed by a rush-desensitization to cow's milk. Ten patients took part in this rush-desensitization starting at 0.1 mg and 9/10 patients reached the final 1,000 mg dose, although one was thereafter treated with epinephrine. Omalizumab was continued until week 16 and two months later, these nine patients tolerated OIT with 4–8 g of milk protein. Serious allergic reactions were seen after 0.1 % of milk doses given. Schneider and colleagues in Boston treated 13 peanut-allergic children (median age 10 years) with omalizumab for 12 weeks,[145] after which all patients passed a rush-desensitization with doses ranging from 0.1 to 500 mg peanut flour. Doses were then further escalated up to 4 g of peanut flour, which was tolerated by 12/13 patients. At this time, the patients stopped omalizumab treatment but were kept on OIT and they all passed an 8 g peanut flour challenge. During treatment, two patients had severe allergic reactions, five had moderate reactions and six had mild or no reactions.

In the last couple of years, two double-blind placebo-controlled studies of OIT (milk and peanut) plus omalizumab or OIT plus placebo have shed more light on the possible positive effects of adjunctive anti-IgE treatment in OIT. In the milk OIT study by Wood et al.,[36] 89 % of the omalizumab-treated subjects (n = 57, age 7–32 years) passed a milk challenge with a cumulative 10 g protein dose after two years of OIT, though this was not significantly higher than the 71.4 % seen in the OIT +placebo group ($p = 0.18$). Omalizumab was stopped at this time, but OIT was continued for eight more weeks. Thereafter, OIT was stopped and the patients were re-challenged after an additional eight weeks. At that time, 48 % in the former omalizumab group and 36 % in the former placebo group passed the same type of challenge ($p = 0.42$). As regards safety, the authors showed significant evidence of substantially lower frequencies of both mild symptoms (2 % vs. 16 %,) and symptoms requiring medical treatment (0 % vs. 3.8 %), for the omalizumab and placebo group, respectively, $p \leq 0.001$.

2.5 IMMUNOLOGIC EFFECTS IN ALLERGEN IMMUNOTHERAPY

The first step towards allergen tolerance in AI is thought to be desensitization of basophils and mast cells. Antigen stimulation of these cells leads to a period of semi-refractory state where sensitivity to allergen stimulation is decreased. The antigen-induced release of tryptase and histamine from mast cells and basophils is also decreased during immunotherapy, decreasing the risk of reaching threshold levels for inducing allergic reactions.[146] Plewako et al. showed that CD203 expression (marker of basophil activation) and basophil production of IL-4 and IL-13 are decreased in the first week of specific immunotherapy for cat allergy.[147] Upregulation of the histamine H2 receptor (HR2) and concomitant suppression of FcεRI-induced basophil activation were demonstrated by Novak et al. in a study of patients undergoing bee venom immunotherapy.[148] Meiler et al. made another HR2-related observation of bee venom-sensitized, but tolerant, bee-keepers. When stung again during a new season, the bee-keepers showed upregulation of the suppressing HR2 on allergen-specific Th2-cells.[149] Although the timing of the immunological events leading to tolerance is not known exactly and varies between patients, some patterns seem quite clear, Figure 4 and 5.[117]

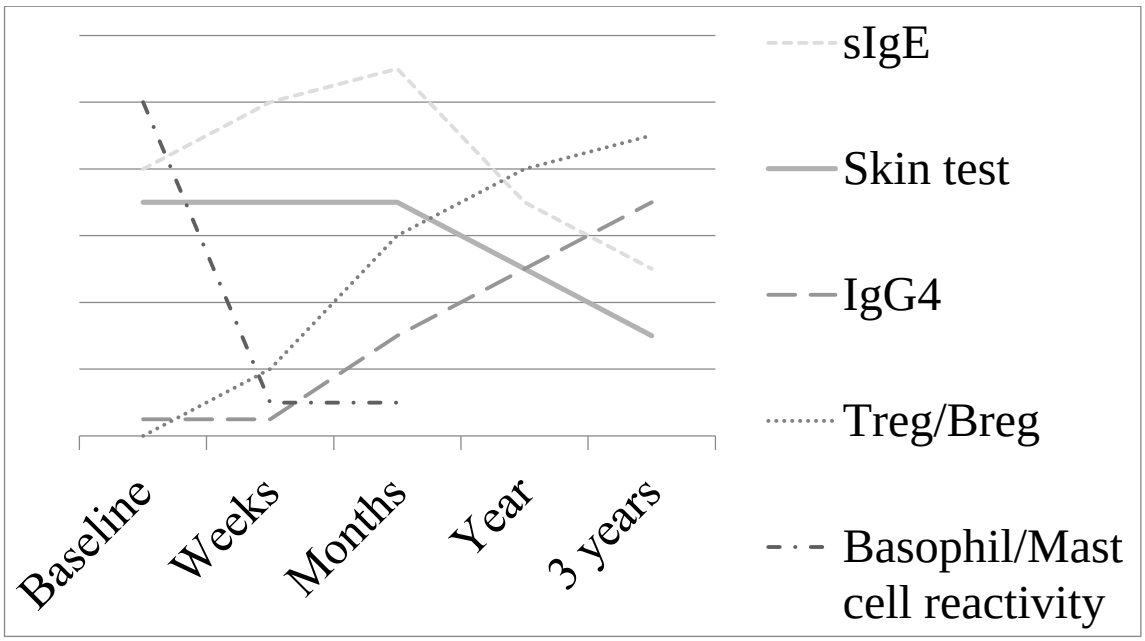

Figure 4. Immunological changes induced by allergen immunotherapy and when these changes occur. The magnitudes of these changes are approximations. Modified from Akdis & Akdis.[117]

2.5.1 Effects on antibodies

Allergen-specific IgE-abs are the initiators of the IgE-mediated allergic reaction. However, there are individuals with high IgE-ab titers who tolerate the allergen of interest and among those who are allergic, severity grade correlates poorly with IgE-ab levels.[150] Despite this, decreasing IgE-ab levels are often seen as allergies spontaneously resolve, as in children who outgrow cow's milk allergy, although measurable quantities are usually still present.[151] In AI, the typical pattern is an initial rise in IgE-ab titers, but IgE-abs to the allergen start to decline after years of treatment.[117] In addition to the previously mentioned protective immunologic mechanisms (such as upregulation of HR2, IL-10, early desensitization of mast cells and basophils), there was a hypothesis of production of blocking antibodies postulated over 80 years ago.[152] These blocking antibodies were later shown to be IgG-abs. The IgG-ab subtype IgG4 has been shown to play an important role in the development of tolerance, both in AI and when allergies are outgrown.[117,151] It also plays a role in tolerant IgE-sensitized individuals; Santos et al. showed that sera from IgE-sensitized peanut-tolerant children that also contained peanut-specific IgG4-abs did not cause the dose-dependent basophil activation response in vitro that was observed when they used sera from peanut-allergic children. After depletion of IgG4 from the peanut-sensitized but tolerant children, a partial basophil activation response was seen and taken as evidence for the protective properties of IgG4.[153] So how do IgG4-abs stop or mitigate IgE-mediated reactions? One theory is that IgG4 binds to FcγR on basophils, and while both inhibitory (FcγRIIB) and activating (FcγRIIA) receptors exist, the inhibitory signals are dominant and they in turn suppress the signals from IgE-FcεRI interaction.[154] Burton et al. provided support for this theory in both mice and humans when they used blocking mAb for the inhibitory FcγRIIB, which resulted in increased IgE-mediated reactivity in vitro in egg-allergic mouse and peanut-allergic children going through immunotherapy.[155] The other proposed mechanism is that IgG4 binds antigens in the tissues in competition with IgE. In a study of children recovering from milk allergy, IgG4 and IgE binding epitopes were shown to overlap. The IgE-antigen bonds were weaker after resolution of allergy compared with baseline values, patients who were still allergic had unaltered IgE-antigen binding.[151] Increased synthesis of IgA-ab in serum and secretory IgA-ab, has also been reported to be associated with successful immunotherapy and the main theory is that IgA works as a gatekeeper in the mucosa

to prevent the allergen from entering the host.[156,157] While increased levels of IgA might be a marker for tolerance development, causal mechanisms are far from proven.

2.5.2 Effects on B and T cells

In a study of IgE-sensitized but tolerant beekeepers, the balance of IFN-γ, IL-10 and IL-4 producing cells (Th1, Treg and Th2, respectively) was altered in favor of IL-10-producing Tregs after being stung.[149] Bee venom allergen-specific B regulatory 1 cells are also increased during immunotherapy.[158] aiTregs (allergen-induced) have been shown to be of importance when allergies are outgrown, as in milk-allergic children who outgrow their allergy.[159] aiTregs have also been shown to increase during oral immunotherapy and an important modulator could be the Forkhead Box Protein 3 (FOXP3). Syed et al. have shown that DNA methylation of the FOXP3 gene is decreased (leading to increased FOXP3 synthesis) during the course of peanut OIT. They also showed that the demethylation was more pronounced in study patients who stayed tolerant to peanuts three months after stopping therapy compared with those who had lost their tolerance. Following the patients for another three months, they found that some additional patients lost their tolerance and this loss of tolerance was associated with an increased FOXP3-methylation. The authors could also show that expression of chemokine receptors such as CCR8 (chemokine c-motif receptor), for which FOXP3 is a transcription factor, increased on aiTregs and that aiTreg migration to intestinal epithelium started to increase after one year of OIT.[160]

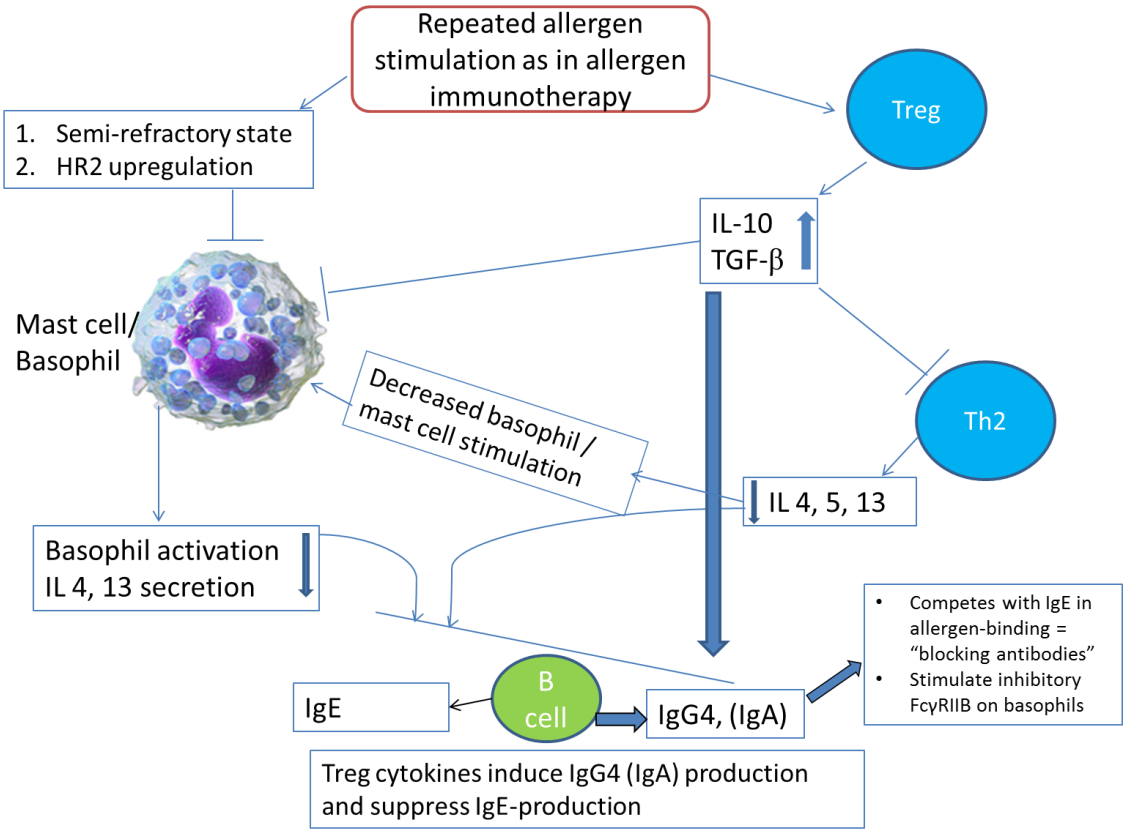

Figure 5. Chain of events induced by allergen immunotherapy. An initial de-sensitization occurs first, as basophils and mast cells get less sensitive to allergen stimulation after repeated exposure allowing for up-dosing. Induction of T-regulatory cells and a subsequent altered balance of cytokine production affect antibody production in favor of protective IgG (IgG4) antibodies.[61,117]

3 AIMS

The overall aim of this thesis was to improve the situation for children diagnosed with allergy to hazelnuts or peanuts. An improvement of the diagnostic accuracy of suspected hazelnut allergy would mean that fewer patients would receive a false positive diagnosis. For children with a severe peanut allergy, we hoped to increase tolerance to peanuts through the use of oral immunotherapy.

Specific aims:

I. To evaluate the diagnostic accuracy of basophil allergen threshold sensitivity (CD-sens) and component-resolved diagnostics in children with suspected hazelnut allergy. (Paper I)

II. To assess whether an effective suppression of peanut allergy can be achieved by an individualized CD-sens monitored omalizumab treatment, where dose and duration are based on repeated CD-sens analyses. To try to find biomarkers or patient characteristics indicative of a need of an elevated omalizumab dose. (Paper II)

III. To evaluate the safety and efficacy of peanut oral immunotherapy combined with individualized omalizumab adjunctive therapy in severely peanut-allergic adolescents. (Paper III)

4 METHODS

4.1 STUDY POPULATIONS

4.1.1 The Hazelnut study (Paper 1)

The study population for the hazelnut study consisted of 40 children aged 6–18 years who had been referred to Sachs' Children and Youth Hospital to go through a hazelnut challenge. The study population consisted of patients with a suspected, but not confirmed, hazelnut allergy, in order to try to get as close to a "real life" clinical setting as possible.

Referral notes were reviewed to assess conformity to the inclusion and exclusion criteria. Thereafter, the patients' caregivers were contacted by phone by a research nurse and asked if they were interested in participating in the study. If the caregivers were interested in participating, written information was sent by mail.

At the time of referral, all patients had to be IgE-sensitized to hazelnut, having either a positive SPT to hazelnut (> 3 mm) or positive IgE to hazelnut (> 0.35 kU_A/l). Further, the patients were required to be on a hazelnut-elimination diet and patients with previous hazelnut-induced anaphylaxis were excluded.

4.1.2 The FASTX study (Papers 2 and 3)

The study population in the FASTX study (Food Allergen Suppression Therapy with Xolair®) consisted of adolescents who had a severe IgE-mediated peanut allergy. The patients were recruited from the outpatient allergy clinic at Sachs' Children and Youth Hospital or referred by pediatric allergists in the Stockholm area. In addition, some patients/caregivers contacted the study team after hearing or reading about the study at clinicaltrials.gov, a website where clinical trials are listed. All patients had a history of dramatic allergic reactions to peanut, but those who had not had an evident peanut-induced anaphylactic reaction within the last five years went through an open peanut challenge prior to inclusion to confirm the severity of the peanut allergy. By only including adolescents with a severe allergy we obtained a homogenous study population. However, the main reason was to offer a potentially disease-modifying treatment to those who would benefit most. When choosing adolescents and not younger children we hoped to achieve: 1. Good ethics; patients participating of their own will. 2. Less troublesome treatment; highly motivated patients who were not afraid of hospitals, injections and blood sampling, and. 3. Reliable reports of adverse events (with young children having a harder time reporting subjective symptoms).

Since the FASTX study did not include a placebo arm, all patients were also required to have a concomitant allergy to either pollen or pets to serve as control. Additional inclusion criteria were positive IgE-test, SPT and CD-sens to peanut and control allergen.

4.2 STUDY DESIGNS

4.2.1 Hazelnut study

We performed double-blind placebo-controlled food challenges (DBPCFC) to hazelnut and compared the outcome with the results from analyses of IgE-abs to hazelnut, the hazelnut components Cor a 1,

Cor a 8, Cor a 9 and Cor a 14 and CD-sens. Tryptase levels were measured before and after the challenge and also ~30 minutes after the onset of symptoms in case of a positive DBPCFC.

4.2.2 FASTX study

The FASTX study was an open one-armed exploratory phase-2 study of peanut oral immunotherapy combined with omalizumab. Laboratory data and patient history were collected at baseline and continuously throughout the study (Table 1). An open peanut challenge for those who had not experienced a peanut-induced anaphylaxis within the last five years was performed at baseline.

	Baseline	Start of OIT	Maintenance	Final visit
Patient history	X			
Peanut challenge	X[a]	X		X[b]
IgE-abs to peanut and Ara h 1, 2, 3, 6, 8, 9	X			X
IgG-abs and IgG4-abs to peanut, Ara h 2 and Ara h 6[c]	X	X	X	X
CD-sens to peanut	X	X	X	X
CD-sens to control allergen	X			X
Skin prick test	X			X[b]

Table 1. Major data collection time points in the FASTX study. [a]If no peanut induced anaphylaxis < 5 years. [b]Only in treatment success patients. [c]Not analyzed for drop-outs.

The FASTX study was divided into two parts. In the first part, omalizumab was started at the dose recommended for asthma. This dose is based on a combination of body weight and total serum IgE. If CD-sens to peanut was negative after eight weeks of treatment (or only reactive at the highest allergen concentration), an open peanut challenge was performed which marked the end of the first part of the study. If a CD-sens value could be calculated, omalizumab was given for another eight weeks with a ~50 % increased dose. This procedure was repeated until CD-sens was suppressed (but not exceeding the maximum recommended dose of omalizumab); thereafter, the open peanut challenge ensued.

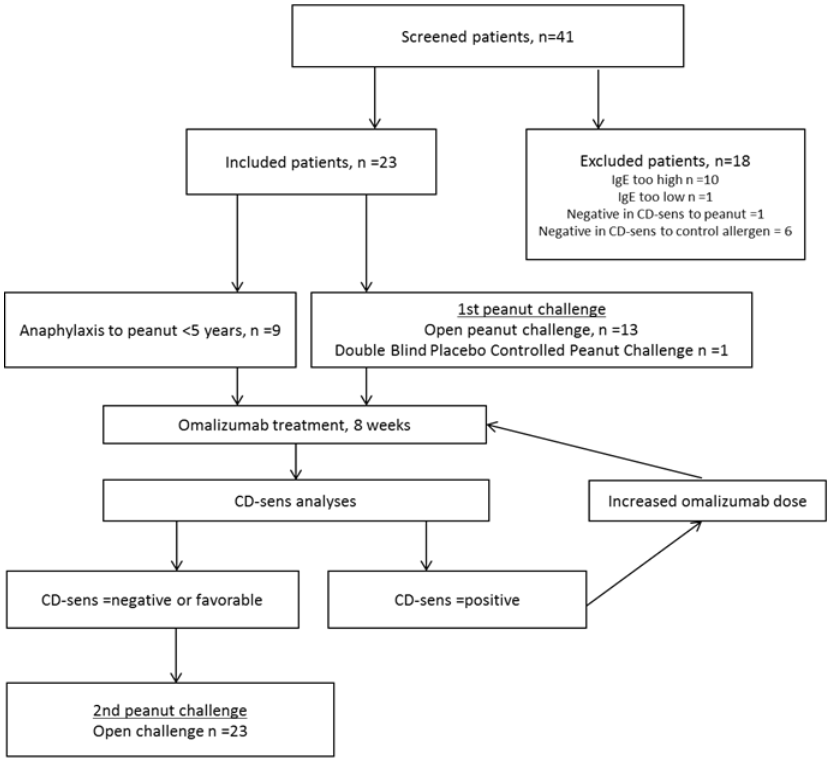

Figure 6. Study design FASTX 1st part.

Next, the second part commenced with peanut oral immunotherapy at a dose of 280 mg of peanut protein, which was increased biweekly until reaching the 2,800 mg maintenance dose. After eight weeks of maintenance doses, we decreased the omalizumab dose (~50 %) if the patient was free of symptoms and CD-sens did not indicate an increased sensitivity to peanuts. This procedure was repeated every eighth week until OIT was tolerated at the lowest omalizumab dose (75 mg every fourth week). At that time, omalizumab was discontinued while OIT continued for 12 more weeks, followed by an open peanut challenge (Figure 7).

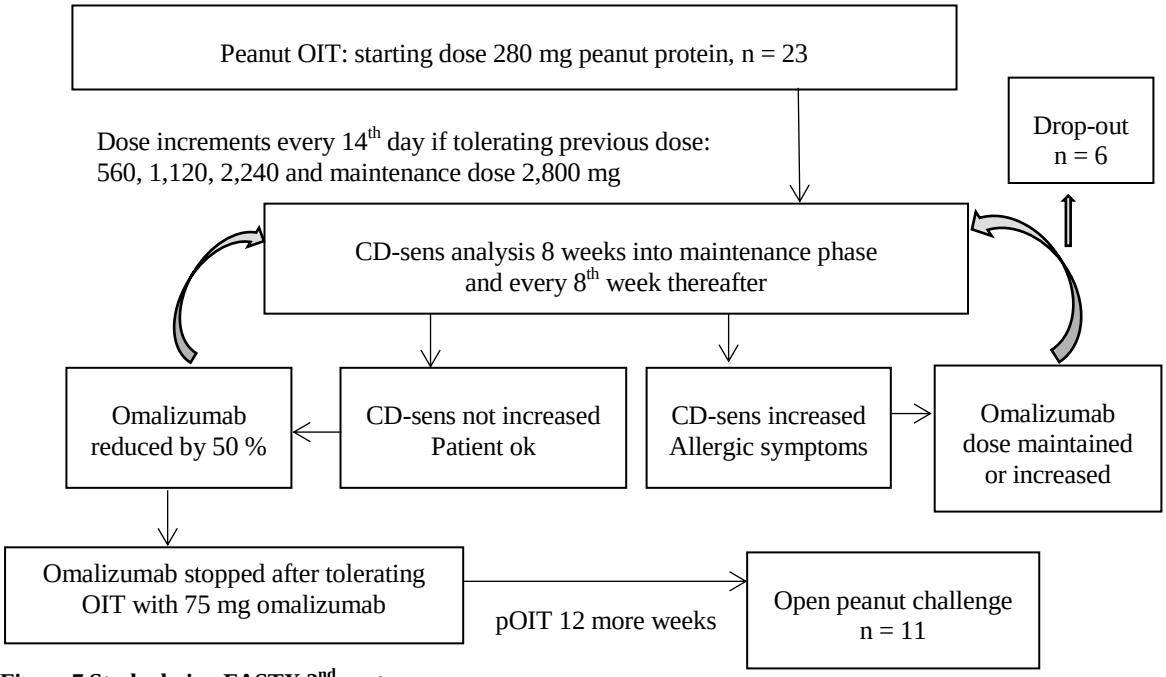

Figure 7 Study design FASTX 2nd part.

4.3 PROCEDURES

4.3.1 Food challenges

In the hazelnut study, double-blind placebo-controlled food challenges (DBPCFC) were used to minimize psychological influence. The challenge medium consisted of chocolate balls containing 11 % raw hazelnuts (omitted in the placebo medium) and was prepared by a dietician using a slightly modified, previously validated, recipe for blind peanut challenges.[161] The challenge consisted of 5 doses of hazelnut: 1 mg, 10 mg, 100 mg, 1 g and 5 g given at 30-minute intervals. The challenge was stopped and ruled positive if the patient had objective symptoms of any severity. Symptom severity was scored as described by Astier.[162]

In FASTX, the challenges were not done to confirm a diagnosis of peanut allergy and therefore we did not find it necessary to perform blinded or placebo-controlled challenges. The purpose of the first challenge was to confirm the severity of the allergy by inducing anaphylaxis. Doses were increased every 30 minutes (0.1, 1 mg, 10 mg, 100 mg, 1 g, and 10 g). The challenge was not stopped in case of, e.g., itching, hives or abdominal pain, but when we were convinced that an anaphylaxis was on the verge of breaking out, the challenge was stopped. Also, doses or dosing intervals could be adjusted at the discretion of the physician. Contrary to common practice, we did not give prompt medication in

the absence of anaphylaxis, but patients were in these cases under very close supervision with emergency treatment drugs readily available.

In the 2nd challenge (just before starting OIT) we aimed to assess how much peanuts the subjects tolerated after the optimized omalizumab dose and confirm that the subjects could tolerate at least the 280 mg peanut protein OIT starting dose. This challenge followed the same dosing intervals as challenge 1. The 3rd challenge, at the final visit, was done to objectively assess that each patient tolerated her/his maintenance OIT dose, post-omalizumab discontinuation, and was performed as a serving of the patient's maintenance pOIT dose.

4.3.2 CD-sens

In the hazelnut study, CD-sens to hazelnut in whole blood was analyzed from blood samples drawn just prior to the first of the two visits for DBPCFC. In FASTX, both whole blood and washed sample CD-sens to peanut were analyzed continuously (8-week intervals) throughout the study, whereas CD-sens to control allergen was analyzed at baseline and at the final visit.

Peripheral blood samples were collected in sterile sodium-heparin collecting tubes. The CD-sens analyses were performed within 24 h (samples stored at +4˚ C pending analysis).[163] The plasma-depleted washed samples were prepared by suspending blood in PBS followed by centrifugation and aspiration of supernatant (twice), followed by resuspension of pellets in RPMI (Rosewell Park Memorial Institute 1640 cell culture medium). Next, both washed and unwashed samples were portioned into tubes and allergen was added. In-house peanut and hazelnut extracts were used and diluted into 8 different concentrations (final peanut protein concentration: 0.83–2,500 ng/ml), (final hazelnut protein concentration: 2-200,000 ng/ml). For control allergens we used commercial extracts (ALK, Copenhagen, Denmark) (final concentrations 5–5,000 SQU/ml). For positive and negative control, we added 25 µl of anti-FcεRI-abs and 100 µl of RPMI, respectively. To be able to identify the basophils and activated basophils, anti-CD63 and anti-CD203c (18 µl) were added to each tube. After 20 minutes incubation, lysing buffer was added (followed by centrifugation and supernatant aspiration) to remove erythrocytes from the samples.

The samples were then analyzed using flow cytometry, where basophils were identified as cells within the 203c+/SSC^{low} (low side scatter) gate and activated basophils as the CD63+ cells within the 203c+/SSC^{low} gate.

4.3.3 IgE and IgG/G4 analysis

Hazelnut study. Samples acquired before the first challenge were stored in -20˚C. At the completion of the study, IgE-abs to hazelnut, Cor a 1, Cor a 8, Cor a 9 and Cor a 14 were analyzed with ImmunoCAP® (Thermo Fisher Scientific, Uppsala, Sweden) by a certified biomedical scientist, in accordance with the instructions from the manufacturer.

FASTX. Blood samples for analyses of IgE-abs to peanut, control allergen and Ara h 1, Ara h 2, Ara h 3, Ara h 8 and Ara h 9 were immediately sent to where samples were analyzed at Karolinska University Hospital Laboratory with ImmunoCAP® (Thermo Fisher Scientific, Uppsala, Sweden), in accordance with the instructions from the manufacturer. We also chose to include analysis of IgE-abs to the peanut component Ara h 6 (baseline and final visit) which was not a part of the initial study protocol. IgE-abs to Ara h 6 were analyzed with ImmunoCAP® (Thermo Fisher Scientific, Uppsala,

Sweden) by a certified biomedical scientist, in accordance with the instructions from the manufacturer.

IgG-abs and IgG4-abs to peanut, Ara h 2, Ara h 6 and control allergen were analyzed at four time points: baseline, start of OIT, eight weeks into maintenance phase and at final visit. Serum was collected at these time points and stored at -20˚C. All analyses were carried out at a single time point by a certified biomedical scientist using the ImmunoCAP® method.

4.3.4 Skin prick test and conjunctival provocation (FASTX study)

SPT and conjunctival provocations were performed by a trained nurse or the author (after receiving training from a trained nurse). For SPT, we used commercial Soluprick® extracts, histamine and diluent were used as positive/negative controls (ALK, Copenhagen, Denmark). Skin prick tests (peanut and control allergen) were performed on all patients at baseline, but only on treatment successes at the final visit since the remaining patients were still being treated with omalizumab, which affects SPT.[164]

Conjunctival provocations (control allergen) were performed at baseline by placing one drop of 100,000 SQU/ml Aquagen (ALK, Denmark) allergen extract in one eye and saline in the other eye as negative control. Symptoms were graded by the physician (0–3) and by the patient on the 0–10 VAS (visual analogous scale). We planned to perform the test at the final visit as well. However, due to unavailability of extracts at the time of final visits, the test could not be performed.

4.4 DATA COLLECTION AND STATISTICS

4.4.1 Data collection

Hazelnut study. Data were continuously collected in electronic medical charts and paper forms by the responsible physician and thereafter entered into the statistical software. CD-sens and IgE-abs were analyzed by staff who were blinded to all patient data, including outcome of the DBPCFC. At the DBPCFC, all involved staff and the patients were unaware of which of the two challenges was with hazelnuts. However, the physician supervising the challenges did have access to previous SPT and/or IgE-ab analyses.

FASTX. We set out to collect all data in a database created for this purpose. However, we soon realized that we had to use the electronic medical charts for collection of data, especially to be able to describe symptoms at food challenges and adverse events during OIT in detail. Patients, physicians and nurses were not blinded to any patient data, while laboratory staff were blinded.

Adverse events, allergic reactions, and other potential adverse events such as illnesses that occurred throughout the study period were reported by the patients or their parents. They reported symptoms by phone or at visits at the clinic. Medical records were reviewed when patients visited the emergency department or were hospitalized. We recorded the frequency of allergic symptoms during pOIT, however, for the two mildest symptoms, oral pruritus and mild abdominal pain, we did not register the exact number of events. All reactions were retrospectively reviewed to assess severity. The allergic reactions were classified as systemic reactions if there was involvement of at least two organ systems. A systemic reaction not fulfilling the World Allergy Organisation (WAO) criteria for anaphylaxis[165] was classified as mild while reactions fulfilling the WAO criteria for anaphylaxis were further

subdivided by the anaphylaxis severity grade as described by Muraro et al. in EAACI guidelines,[166] into moderate, anaphylaxis grade 1–2, and severe, anaphylaxis grade 3.

4.4.2 Statistical analysis

In the hazelnut study, GraphPad Prism 5.01 (GraphPad Software Inc. Ca, USA) was used for all statistical analyses and in FASTX we used STATA 14.0 (Stata Corp, TX, USA). For all papers included in this thesis, p values < 0.05 were considered significant (no adjustments for multiple analyses were made).

In paper 1, the hazelnut study, we compared IgE-ab levels and CD-sens to hazelnut in relation to outcome of DBPCFC. Data were reported as medians and $25^{th}/75^{th}$ percentiles and the Wilcoxon rank-sum test was used when comparing groups as data were not normally distributed. ROC curves (receiver operating characteristic curves) where constructed to find the cut-off levels for IgE-abs/CD-sens with the highest possible sensitivity and specificity.

In the first FASTX study (paper 2) we reported laboratory data and other continuous variables as medians and $25^{th}/75^{th}$ percentiles. Binomial variables were presented as percentages. For statistical comparison of the two groups (normal dose (omalizumab) and elevated dose) we used Fisher's exact test for binomial variables and Wilcoxon rank-sum test for continuous variables (in the published version of this paper we wrongly state that Fisher's test was used for both). ROC calculations were also performed on baseline predictors of outcome.

In the 2^{nd} FASTX study (Paper 3) we looked for changes over time in IgE/IgG-ab levels, CD-sens, and SPT. In these serial observations we used the non-parametric Page's test for trends and the sign-rank test for comparison of two time points. Descriptive statistics were carried out using Fisher's exact test, Wilcoxon rank-sum test (comparing two groups) and Kruskal-Wallis test (comparing 3 groups at once).

4.5 ETHICS AND REGISTRATION

All studies within this thesis were approved by the Stockholm Ethics Committee (Hazelnut study; 2012/990-31/3, FASTX; 2013/827-31/3 and two amendments that were approved 20 November 2014 and 13 December 2015, respectively). The FASTX study was also approved by the Swedish Drug Agency (5.1-2013-46183). All caregivers of the patients in these studies (or the patient if > 18 years of age) provided written informed consent. The FASTX study was registered at ClinicalTrials.gov; NCT02402231 and EudraCT; 2012-005625-78.

5 RESULTS

Results are in general reported as: median (25^{th}-75^{th} percentile) and if not so it is stated.

5.1 FOOD CHALLENGES (PAPERS 1-3)

In the hazelnut study, only 8/40 (20 %) of the patients, who all avoided hazelnuts due to suspected hazelnut allergy (based on patient history and previous diagnostic work-up), reacted with objective symptoms at food challenge. This means that 80 % of the patients had either received an incorrect diagnosis or had outgrown their allergy. There were no observed reactions in the 40 placebo challenges. Another interesting finding was that in ¼ of the positive challenges (5 % of all DBPCFCs) the patient was admitted to the pediatric ward for continued observation due to severe symptoms.

In the FASTX study, we performed the 1^{st} peanut challenge to confirm the severity of each subject's peanut allergy. This challenge was performed on 13/23 patients (one patient had gone through a DBPCFC within another study). The median dose ingested at this challenge was 91 mg peanut protein, but symptoms usually presented at lower doses (dose escalation was not stopped for subjective symptoms or mild objective symptoms). Of the challenged patients; 9/14 received ≥ 1 dose of adrenaline, four were hospitalized and two of them had bi-phasic reactions with recurring respiratory symptoms. All 23 patients participated in the 2^{nd} peanut challenge that took place once we observed omalizumab-induced suppression of CD-sens to peanut. Even if the patient tolerated the maximum 2,800 mg peanut protein dose, the challenge was stopped at this dose. Table 2 summarizes objective symptoms observed in these challenges and also at the final peanut challenge which was performed 12 weeks after stopping omalizumab (while still being on peanut OIT).

	Before omalizumab (n = 14)	**With omalizumab (n = 23)**	**After omalizumab and OIT (n = 11)**
Objective symptoms			
Vomiting	7 (50 %)	0	0
Urticaria	6 (43 %)	1 (4 %)	0
Conjunctivitis	6 (43 %)	4 (17 %)	0
Asthma	4 (29 %)	0	0
Rhinitis	4 (29 %)	3 (13 %)	0
Cough	3 (21 %)	0	0
Erythrodermia	3 (21 %)	1 (4 %)	0
Muffled voice	3 (21 %)	0	0
De-saturation (< 90 %)	1 (7 %)	0	0
Stridorous breathing	1 (7 %)	0	0
Adrenaline ≥ 1 dose	9 (64 %)	0	0
Adrenaline ≥ 2 doses	4 (29 %)	0	0
Adrenaline 3 doses	1 (7 %)	0	0
Peanut protein dose in mg			
Median (min-max)	91 mg (28–840)	2,800 (840–2,800)	2,800 (700–2,800)

Table 2. Objective symptoms, adrenaline treatment and ingested peanut dose at peanut challenges 1, 2 and 3 (1; at baseline in those subjects without history of anaphylaxis within the last five years, 2; while on omalizumab after CD-sens to peanut was suppressed, 3; while on OIT after discontinuing omalizumab).

5.2 DIAGNOSTIC MARKERS FOR HAZELNUT ALLERGY (PAPER 1)

5.2.1 IgE-abs to hazelnut and hazelnut components

The study population had high titers of total serum IgE with a median of 423 kU/l (230–996). Sensitization to hazelnut was present in 39/40 patients; median 9.75 kU_A/l (3.73–36.2) and did not differ significantly in those with confirmed hazelnut allergy, median 11.4 (6.51–79.8), and in those tolerant to hazelnuts, median 9.2 (3.03–34.9) (P = 0.34). Half of the patients in both groups had a hazelnut IgE < 10 kU_A/l. Tolerant patients had higher IgE-ab levels to Cor a 1 (cross-reactive with birch) and birch; three of eight hazelnut-allergic patients had no IgE-abs to birch at all (median 0.82 kU_A/l (0–7.56)), while among the tolerant patients 31/32 were birch-sensitized (median 12.6 kU_A/l (3.12–63.8)) (P = 0.013).

All hazelnut-allergic patients were sensitized to both Cor a 9 and Cor a 14. Cor a 14 IgE-abs differed most between allergic and tolerant patients; median 5.6 kU_A/l (0.9–78.7) for allergic, median 0.04 kU_A/l (0–13.9) for tolerant (P < 0.001). Median IgE-ab levels to Cor a 9 were 4.5 kU_A/l (0.7–97) and 0.1 kU_A/l (0–36.2) in hazelnut-allergic and in tolerant patients respectively (P < 0.01).

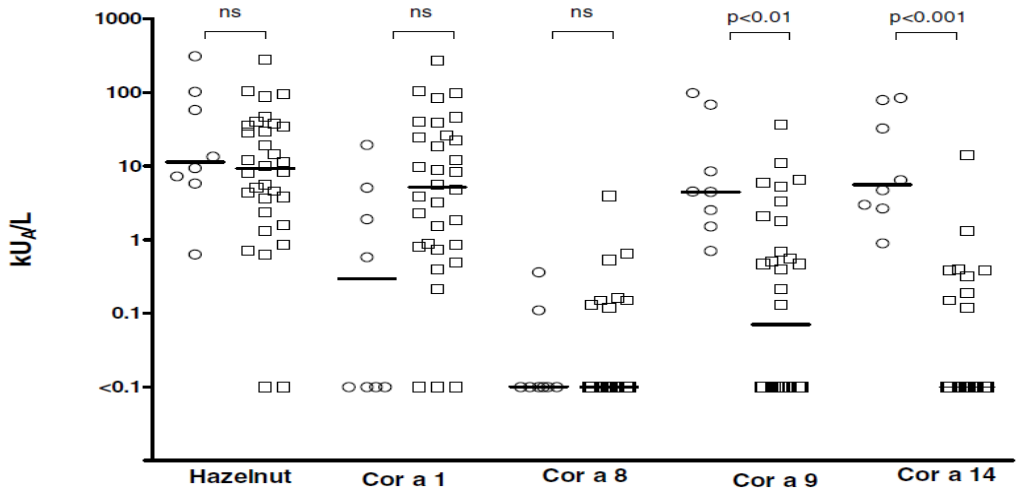

Figure 8. IgE-abs to hazelnut and hazelnut components. ○=hazelnut-allergic, □= hazelnut-tolerant.

ROC curves were generated and for Cor a 14 a sensitivity and specificity of 100 % and 94 % respectively at a cut-off of 0.64 kU_A/l was reached, while the corresponding figures for Cor a 9 was 100 % sensitivity with 72 % specificity (cut-off 0.65 kU_A/l). The 95 % confidence intervals for the area under the curve were overlapping for the Cor a 14 and Cor a 9 ROC curves (Figure 9).

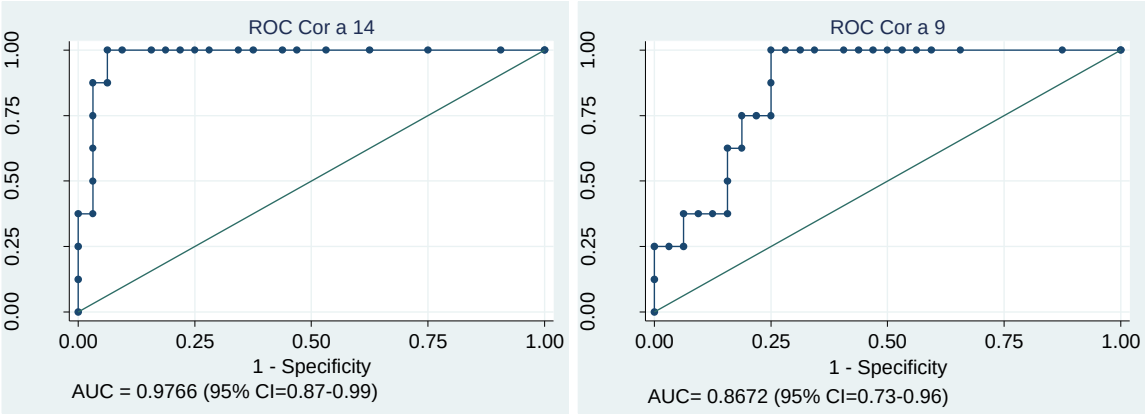

Figure 9. ROC curves for IgE-abs to Cor a 14 and Cor a 9.

5.2.2 CD-sens

CD-sens to hazelnut had a sensitivity of 100 %, since all patients with a positive DBPCFC were positive in CD-sens to hazelnut. While most (24/32) tolerant patients had a positive CD-sens, their values were, with one exception, much lower. Median CD-sens in hazelnut-allergic patients was 8.9 (5.2–41), while tolerant patients had a median of 0.05 (0.007–0.11), making for a highly significant difference, $P < 0.0001$. At a ROC-generated cut-off of CD-sens ≥ 1.7, a sensitivity of 100 % and specificity of 97 % was reached (Figure 10).

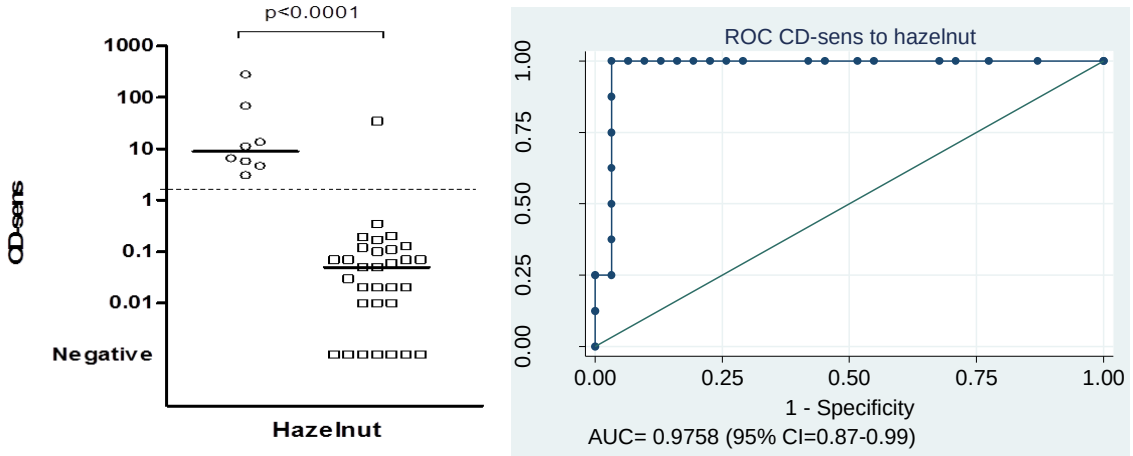

Figure 10. a. CD-sens to hazelnut among hazelnut-allergic patients= ○ and patients tolerant to hazelnut at DBPCFC=□. b. ROC curve for CD-sens to hazelnut in relation to outcome of DBPCFC.

5.3 FASTX (PAPERS 2 AND 3)

Forty-one patients were assessed for eligibility and 23 matched the inclusion criteria. The main reason for being excluded was too high total serum IgE (in 10/18 non-included subjects). However, there were no limitations in IgE-abs to peanut, possibly resulting in patients with high ratios of allergen-specific IgE-abs to peanut (and peanut components) in relation to total IgE. Six patients were not included since they were negative to the control allergen in CD-sens.

Table 3 reports patient characteristics of the whole study population in papers II and III (patient characteristics in paper II are reported in relation to if the subject received an increased omalizumab dose or not, while in paper III they are reported in relation to outcome of peanut OIT).

Patient characteristics	n (%)
Gender, female	16 (70)
Conjunctivitis	21 (91)
Asthma	20 (87)
Rhinitis	20 (87)
Eczema	6 (26)
Other food allergy	14 (61)
Allergic to tree/grass pollen	20 (87)
Allergic to pets	18 (78)
> 2 atopic manifestations	15 (65)
	Median (25^{th}–75^{th} percentile)
Skin prick test peanut, mm	10 (9–14)
Total serum IgE, kU/l	470 (230–680)
Peanut, kU_A/l	86 (48–220)
Ara h 2, kU_A/l	58 (26–78)
Ara h 6, kU_A/l	49 (26–66)
Ara h 8, kU_A/l	2.3 (0.61–6.6)
Ara h 2/total IgE	14.4 % (10.6–20)
CD-sens peanut	0.8 (0.4–1.9)
Omalizumab start dose (mg per 4 weeks)	600 (150–1,050)
Age, years (min-max)	17 (12–19)

Table 3. Patient characteristics of the study population in papers II and III.

5.3.1 Dosage of omalizumab

After eight weeks of treatment with omalizumab (at doses recommended for asthma) CD-sens was suppressed in 8/23 patients (CD-sens was completely negative or showed a small reaction at the highest allergen concentration only). Among twelve of the remaining 15 patients, basophils were suppressed after eight more weeks of treatment with omalizumab with an increased dose. In another patient, basophil suppression was reached at week 24, while one patient (who already had been on the maximum allowed dose since week 8) was still barely positive in CD-sens but was allowed to move on to the peanut challenge. The last patient needed 32 weeks of omalizumab treatment, with a final dose 400 % higher than the starting dose in order to suppress CD-sens to peanut (Figure 11).

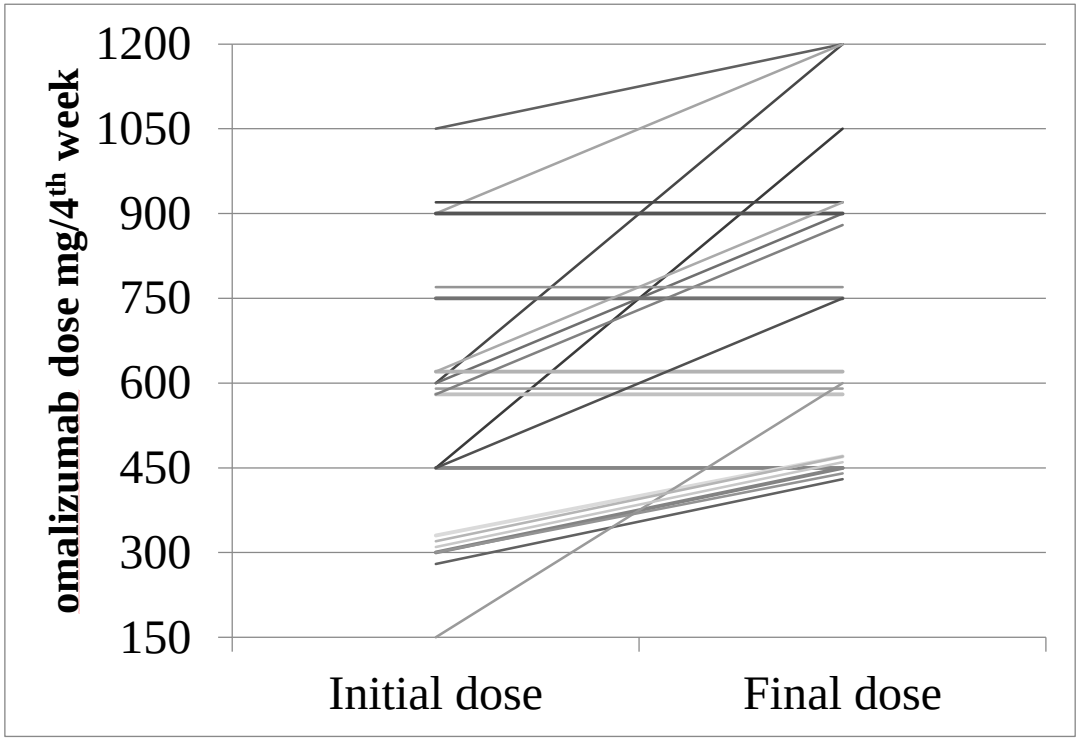

Figure 11. Initial and final dose of omalizumab. Each line represents a patient; when patients have identical starting and final doses the lines are slightly offset from the actual values they represent; e.g., when the graph shows value of 580, the actual value is 600.

5.3.2 Who needs an increased omalizumab dose?

In terms of medical history of atopic diseases and age, there were neither any significant differences nor any interesting trends in relation to a need for an increased omalizumab dose. Patients who needed an increased omalizumab dose had higher CD-sens to peanut, median 1.5 (0.61–2.7), compared with those who did not increase their dose, 0.32 (0.28–0.55). Total serum IgE was markedly higher (in median 660 kU/l) among those whose basophil response to peanut was suppressed after eight weeks of omalizumab (at a normal asthma dose) compared with 260 kU/l ($P < 0.01$) among the subjects who needed an elevated dose. Also the IgE-ab levels to peanut and peanut components were lower (not significantly) among those who needed an increased omalizumab dose. The ratio of Ara h 2 IgE/Total IgE was in median 17.1 % (5.8–50 %) in the group who needed an increased omalizumab dose; significantly higher than the median ratio of 10.9 % (5.2–15.4) in the normal omalizumab group ($P = 0.02$).

5.4 PEANUT ORAL IMMUNOTHERAPY (POIT)

The immunotherapy in FASTX consists of three phases. The first two were the up-dosing phase and the maintenance phase (during this phase omalizumab treatment was gradually phased out) (reported in paper III). In the third phase, which is still ongoing, OIT is given for an additional three years (without omalizumab). During up-dosing and maintenance, approximately 16,000 pOIT doses were administered.

Up-dosing phase: After the 2nd peanut challenge (where all patients ingested > 3 g of peanuts), pOIT was initiated at home the following day with 1 g of peanut ~280 mg peanut protein. During up-dosing at home, two patients had moderate adverse events while on this first dose step (1 g); both occurring

after physical exercise. These were the only systemic reactions observed during up-dosing (roughly 1,860 pOIT doses were administered). Most patients experienced mild oral itching or mild abdominal pain during up-dosing, depending on frequency and intensity this affected how fast the up-dosing could be performed. The maintenance dose of 10 g of peanuts was reached in a median of ten weeks (8–13 weeks). However, it turned out that the subjects who later successfully completed the study reached the maintenance dose faster, in eight weeks (median), compared with those who could not successfully discontinue omalizumab, needing 14 weeks to reach maintenance dose. Patients who later dropped out of the study were in between (11.5 weeks). No patients dropped out during up-dosing.

Maintenance phase: Dose reduction of omalizumab was considered every 8th week. There was a wide variance of the rate that omalizumab could be decreased and it became apparent after two years that some patients might not be able to discontinue omalizumab in a near future. Therefore we decided, at an investigator's meeting in March 2016, that patients still treated with omalizumab in September 2017 should be considered treatment failures. Treatment successes received pOIT for a median time of 83 weeks (min-max 48–156), while the treatment failures (still on omalizumab) had been on pOIT for 139 weeks (115–166). Among the drop-outs, the median time of pOIT was 72 weeks (min-max 38–120).

The primary end-point, passing the 10 g peanut challenge after tolerating pOIT with 10 g of peanuts for 12 weeks after the omalizumab treatment was stopped, was met by 9/23 (39 %) subjects. Two more patients also completed the study, but with a reduced pOIT dose (~2.4 and 4 grams), as they had developed a profound abomination for the taste of peanuts. Study completion with any pOIT dose was met by 11/23 (48 %) of the subjects. When excluding drop-outs (per-protocol analysis), 11/17 (65 %) successfully completed the study.

In all but two patients either adverse events or an increasing CD-sens value made us postpone the down-escalation of one or more omalizumab doses.

5.4.1 Adverse events

There were 43 systemic reactions attributed to pOIT during the FASTX study. One was classified as severe, 22 as moderate and 20 as mild. A vast majority (19/22) of moderate systemic reactions were graded as moderate based on reports of subjective breathing difficulties in combination with symptoms from the skin or mucosal tissues. The frequency of systemic reactions was one per 374 pOIT doses ingested (0.3 %), but the cumulative incidence was 70 % (16/23) and 43 % were treated with adrenaline at least once. Patients who dropped out (n = 6) did not have more frequent allergic reactions than the other patients (n = 17) and in general had fewer episodes with allergic symptoms than treatment failures. One patient developed eosinophilic esophagitis. Table 4 shows detailed information of allergic AEs among treatment successes and treatment failures.

The study design does not allow us to make causal inference as to whether omalizumab decreases the risk of AEs or not, but two interesting observations can be reported: Of the 43 systemic reactions, only four occurred while the subject was on full dose omalizumab, and among treatment failures, 22/27 systemic reactions occurred when the omalizumab dose was reduced to ≤ 25 % of the original dose.

	Total (n = 17)			Treatment success (n = 11)			Treatment failure (n = 6)		
Total peanut doses	12,915			6,978			5,937		
Peanut doses per child, mean (range)	760 (336–1,163)			634 (336–1,093)			990 (804–1,163)		
	Patients with event[b] n (%)	Mean (range) for patients with event[b]	Doses with event[b] n (%)	Patients with event[b] n (%)	Mean (range) for patients with event[b]	Doses with event[b] n (%)	Patients with event[b] n (%)	Mean (range) for patients with event[b]	Doses with event[b] n (%)
SYMPTOMS									
Skin									
Urticaria	7(41.2)	1.9(1–3)	13(0.1)	3(27.3)	1.3(1–2)	4(0.1)	4(66.7)	2.3(1–3)	9(0.2)
Erythema-flush	1(5.9)	6.0(6)	6(0.0)	0	-	0(0)	1(16.7)	6.0(6)	6(0.1)
Angioedema	2(11.8)	2.0(1–3)	4(0.0)	1(9.1)	1.0(1)	1(0.0)	1(16.7)	3.0(3)	3(0.1)
Pruritus	9(52.9)	2.8(1–8)	25(0.2)	4(36.4)	2.0(1–3)	8(0.1)	5(83.3)	3.4(1–8)	17(0.3)
Gastrointestinal									
Vomiting	2(11.8)	1.5(1–2)	3(0.0)	1(9.1)	1.0(1)	1(0.0)	1(16.7)	2.0(2)	2(0.0)
Severe abd. pain	5(29.4)	1.2(1–2)	6(0.0)	3(27.3)	1.3(1–2)	4(0.1)	2(33.3)	1.0(1)	2(0.0)
Respiratory									
Conjunctivitis	2(11.8)	1.0(1)	2(0.0)	1(9.1)	1.0(1)	1(0.0)	1(16.7)	1.0(1)	1(0.0)
Rhinitis	4(23.5)	6.3(1–21)	25(0.2)	1(9.1)	1.0(1)	1(0.0)	3(50.0)	8.0(1–21)	24(0.4)
Cough	1(5.9)	1.0(1)	1(0.0)	0	.	.	1(16.7)	1.0(1)	1(0.0)
Subj. throat tight.	8(47.1)	1.8(1–4)	14(0.1)	3(27.3)	1.3(1–2)	4(0.1)	5(83.3)	2.0(1–4)	10(0.2)
Chest tightness	2(11.8)	1(1)	2(0.0)	0	-	0(0*)	2(33.3)	1.0(1)	2(0.0*)
Subj. breath. diffic.	9(52.9)	2.8(1–8)	25(0.2)	4(36.4)	1.4(1–4)	8(0.1)	5(83.3)	3.4(1–8)	17(0.3)
Wheeze	0(0)	-	0(0)	0	-	0(0)	0	-	0(0)
De-sat < 92 %)	1(5.9)	1.0(1)	1(0.0)	1(9.1)	1.0(1)	1(0.0)	0	-	0(0)
Neuro-cardiovasc.									
Chest pain	2(11.8)	2.0(2)	4(0.0)	1(9.1)	2.0(2)	2(0.0)	1(16.7)	2.0(2)	2(0.0)
Dizziness	2(11.8)	1.0(1)	2(0.0)	1(9.1)	1.0(1)	1(0.0)	1(16.7)	1.0(1)	1(0.0)
Syncope	1(5.9)	1.0(1)	1(0.0)	1(9.1)	1.0(1)	1(0.0)	0	-	0(0)
Hypotension	0	-	0(0)	0	-	0(0)	0	-	0(0)
SYSTEMIC REACTIONS[a]	12(70.6)	3.1(1–14)	37(0.3)	6(54.6)	1.7(1–3)	10(0.1)	6(100)	4.5(1–14)	27(0.5)
Mild[c]	8(47.1)	2.1(1–7)	19(0.1)	5(45.5)	1.2(1–2)	6(0.1)	3(50)	4.3(2–7)	13(0.2)
Moderate [d]	7(41.2)	2.4(1–7)	17(0.1)	2(18.2*)	1.5(1–2)	3(0.0*)	5(83.3*)	2.8(1–7)	14(0.2*)
Severe [e]	1(5.9)	1(1)	1(0.0)	1(9.1)	1(1)	1(0.0)	0	-	0(0)
DRUGS adm.									
i.m. adrenaline	8(47.1)	1.9(1–4)	15(0.1)	3(27.3)	1.7(1–3)	5(0.1*)	5(83.3)	2(1–4)	10(0.2*)
At home	7(41.2)	1.9(1–3)	13(0.1)	2(18.2*)	2(1–3)	4(0.1*)	5(83.3*)	1.8(1–3)	9(0.2*)
In ambul/hospital	2(11.8)	1(1)	2(0.0)	1(9.1)	1(1)	1(0.0)	1(16.7)	1(1)	1(0.0)
Repeated doses	1(5.9)	1(1)	1(0.0)	0	-	-	1(16.7)	1(1)	1(0.0)
Inhaled b-2 agonist	7(41.2)	1.7(1–3)	12(0.1)	3(27.3)	1.7(1–3)	5(0.1)	4(66.7)	1.8(1–2)	7(0.1)
At home	7(41.2)	1.7(1–3)	12(0.1)	3(27.3)	1.7(1–3)	5(0.1)	4(66.7)	1.8(1–2)	7(0.1)
In ambul/hospital	1(5.9)	1(1)	1(0.0)	1(9.1)	1(1)	1(0.0)	0	-	0(0)
Intravenous fluid	2(11.8)	1(1)	2(0.0)	2(18.2)	1(1)	2(0.0)	0	-	0(0)
ED visit	7(41.2)	1.9(1–3)	13(0.1)	3(27.3)	1.7(1–2)	5(0.1)	4(66.7)	2.0(1–3)	8(0.1)
Hospital admission	2(11.8)	1(1)	2(0.0)	0	-	0(0*)	2(33.3)	1(1)	2(0.0*)

Table 4. Peanut doses, allergic symptoms, administered medical therapy and ED-visits in relation to outcome. [a]Systemic reaction = dose-related allergic reaction with involvement of ≥ 2 organ systems, [b]Event = peanut dose related symptom, systemic reaction[a], administered therapy or ED-visit, [c]Mild systemic reaction = systemic reaction but not anaphylaxis, [d] Moderate systemic reaction = anaphylaxis grade 1–2, [e] Severe systemic reaction = anaphylaxis grade 3, * TF vs. TS: p value < 0.05. Abbreviations: abd. =abdominal, de-sat=desaturation, i.m.=intramuscular, Subj. breath. diffic.= subjective breathing difficulties

5.4.2 Drop-outs

Six patients dropped out of the study. These six patients did not differ from the other study participants in terms of baseline levels of IgE-abs, CD-sens or patient history, neither did they experience worse or more frequent allergic reactions. One of the patients had almost completed the study (omalizumab had been stopped) when a gastroscopy performed as part of the diagnostic work-up of long-lasting symptoms (chronic cough and difficulties swallowing certain foods) revealed that he suffered from eosinophilic esophagitis. He stopped pOIT and his symptoms resolved completely. The other five patients who dropped out did so for personal reasons, fear of having allergic reactions, lack of motivation and moving/travelling, they also highly disliked the taste of peanuts which contributed to their decision.

5.5 IMMUNOLOGY

5.5.1 Predictors of outcome

Just as in the first FASTX paper (paper II), where a high CD-sens indicated a need for a higher omalizumab dose, pOIT treatment failures had higher CD-sens to peanut at baseline with a median of 6.7 (1.5–9.5) compared with the treatment successes whose median was 0.52 (0.29–1.5) (P = 0.021). IgE-abs to peanut and peanut components Ara h 1, 2 and 3 were also significantly higher among those failing the pOIT protocol, e.g., Ara h 2: 72 kU_A/L (58–185) vs. 30 kU_A/L (24–66) (P = 0.034).

5.5.2 Immunological changes

IgE-ab levels to peanut, peanut components and control allergen at the end of the study were not significantly different compared with the baseline levels. However, the SPT reaction to peanut decreased significantly in the successfully treated patients from a baseline median of 12 mm to 7 mm at final visit (P < 0.01) while SPT to the control allergen showed a slight, non-significant decline from 7 mm to 5 mm (P = 0.1).

IgG-ab and IgG4-ab production to peanut (and peanut components) was induced by pOIT but not by omalizumab. From baseline to the start of pOIT IgG/G4-titers remained literally constant, but increased markedly after the up-dosing phase in all patients. Also, IgG/G4 levels to control allergens remained very stable throughout both omalizumab and pOIT.

During maintenance phase, however, the successfully treated patients had a significantly higher increase in IgG4 to peanut, Ara h 2 and Ara h 6. Final levels of IgG4-abs to Ara h 2 among adolescents with treatment success were 6 times higher than at start of the maintenance phase (P < 0.01), whereas the corresponding increase for treatment failures was only 1.3-fold (P = 0.35).

The allergen-binding activity (ABA) increased significantly from a baseline 3.8 (2.2–5.9) to a final visit value of 49 (9–89) (P < 0.01) among the successfully treated subjects. We also observed a positive correlation between ABA and IgG4 to peanut (rho = 0.67, P = 0.0499), to Ara h 2 (rho = 0.70, P = 0.036) and to Ara h 6 IgE-abs (rho = 0.70, P = 0.036) at the end of the study. In these analyses of ABA and correlations between ABA-IgG4 we had to exclude two patients who had turned into "non-responders" in CD-sens (non-reactive in CD-sens to peanut, control allergen and positive control (FcεRI-stimulation).

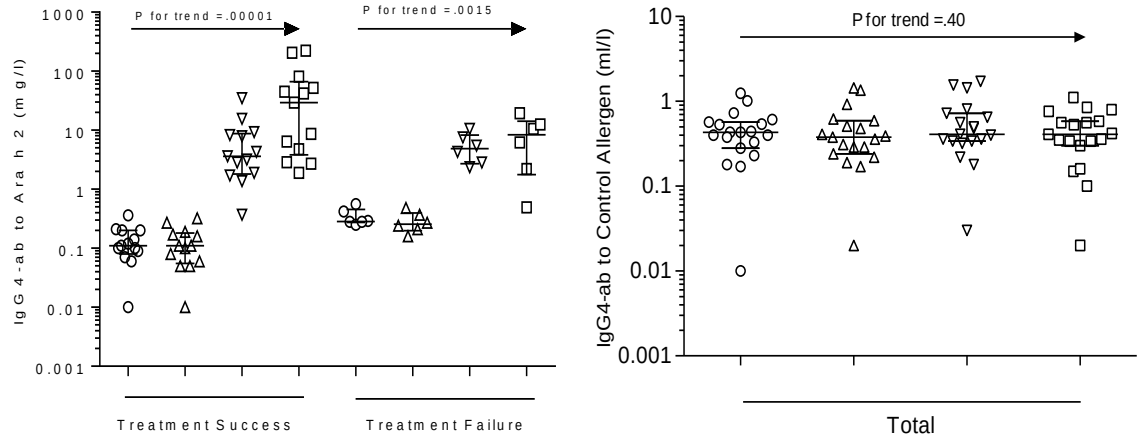

Figure 12. IgG4-ab levels at: ○ Baseline, Δ Start of OIT, □ Maintenance Phase, □ Final Visit. a; Ara h 2, b; Ara h 6, c; Control allergen. Error bars are presented as median (inter quartile range). A statistical significance was considered at a p value of < 0.05.

5.6 UNPUBLISHED DATA

5.6.1 Subgroup analysis of CD-sens in hazelnut diagnostics study

There were 23 patients in the hazelnut study who had an IgE-ab level of > 0.35 kU_A/l to Cor a 9 or Cor a 14 (15 tolerant and all 8 allergic patients). In a kind of sensitivity analysis, the ability of CD-sens to discriminate between allergy and tolerance was assessed when patients below different cut-off points for Cor a 9/Cor a 14 were excluded from analyses (Table 5).

	Tolerant	**Allergic**	***P***
All patients	n = 32	n = 8	
CD-sens, median (min-max)	0.05 (0–34.7)	8.9 (3.1–281)	< 0.0001
Cor a 9/Cor a 14 > 0.35 kU_A/l	n = 23	n = 8	
CD-sens, median (min-max)	0.02 (0–34.7)	8.9 (3.1–281)	0.0005
Cor a 9/Cor a 14 > 2 kU_A/l	n = 8	n = 7	
CD-sens, median (min-max)	0.04 (0–0.2)	6.6 (4.7–281)	0.0012

Table 5. CD-sens in relation to outcome in DBPCFC in patients with IgE-sensitization to Cor a 9/Cor a 14 above the two cut-points 0.35 and 2.0 kU_A/l

5.6.2 CD-sens in relation to adverse events

As stated previously; if the patients had been clinically tolerant for ≥ 2 eight-week cycles, the omalizumab dose could be decreased despite a calculable (but non-increasing) CD-sens to peanut. For the vast majority of pOIT doses consumed, when the most recent CD-sens was calculable, no adverse events occurred. However, among the systemic reactions there were three moderate reactions in spite of the last CD-sens being negative to peanut, while 19 moderate and the only severe reaction occurred when the most recent CD-sens was positive.

5.6.3 Long-term pOIT without omalizumab

The eleven patients who successfully completed the FASTX study were invited to continue pOIT and participate in a follow-up study of pOIT for another three years. All 11 patients agreed and seven of them are still under pOIT while 4 have decided to stop the treatment. We have data from follow up visits after one year of OIT without omalizumab (n = 5) and after two years of OIT without omalizumab (n = 2). These five patients had very few adverse events throughout the FASTX study and they continue to tolerate the treatment well with a frequency of potential systemic reactions of around 1 per 2,000 doses. At the 1-year follow up, IgE-ab levels to both peanut and Ara h 2 had declined to below the baseline levels. Figure 13 shows changes in IgE-ab levels over time.

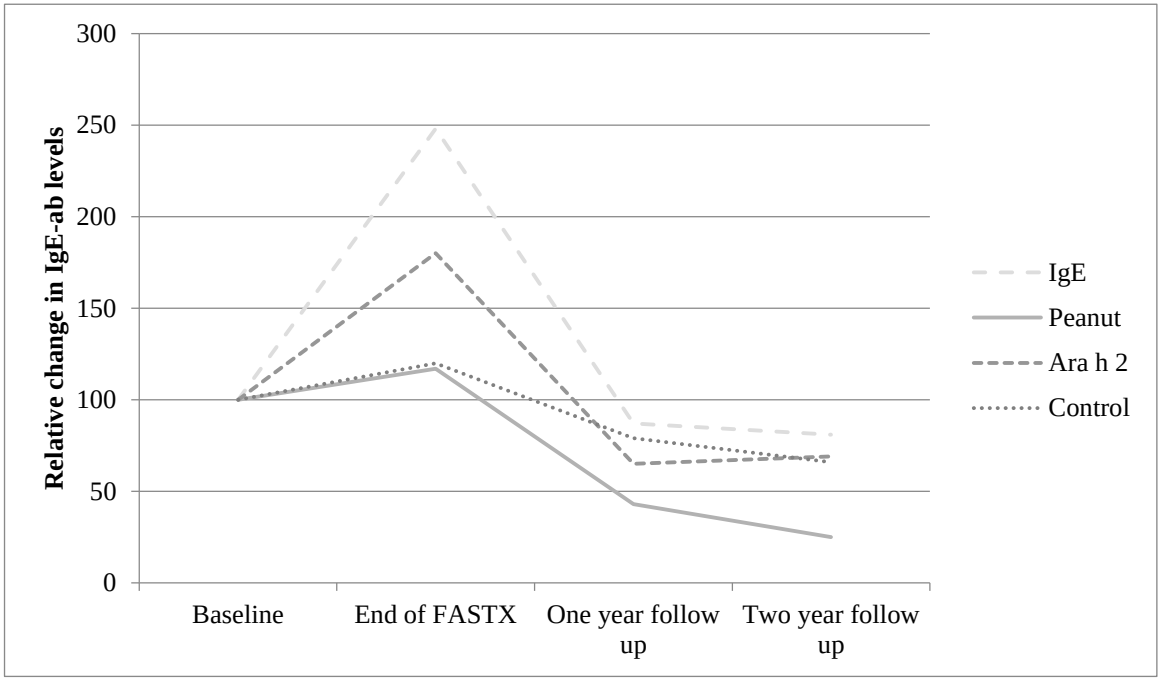

Figure 13. Total IgE and allergen-specific IgE-abs to peanut, Ara h 2 and control allergen for subjects completing 1 more year of OIT after finishing the FASTX study (n = 5) and for those completing two additional years (n = 2).

6 DISCUSSION

6.1 HAZELNUT ALLERGY DIAGNOSTICS STUDY

The problem when diagnosing hazelnut allergy is the low specificity of the diagnostic tests, in particular among patients with birch pollen allergy, who often experience oral allergy syndrome upon ingestion of hazelnuts.[167-169]

We showed that both CD-sens to hazelnut and component-resolved diagnostics with analysis of IgE-abs to Cor a 9 and 14 were far superior in terms of specificity to the previously available serological tests for hazelnut allergy (IgE to hazelnut, Cor a 1) and this was without a trade-off to lost sensitivity (which is generally the case with diagnostic tests): all patients with a positive DBPCFC to hazelnut had a positive CD-sens and were IgE-sensitized to both Cor a 9 and Cor a 14 with IgE-ab levels above 0.35 kU_A/l. However, despite the perfect sensitivity of these tests in this study, there are several cases of hazelnut-allergic patients in other studies who are not sensitized to either Cor a 9 or Cor a 14, especially among adults.[15,17,104]

Our data indicate that IgE-sensitization to Cor a 14 was superior to IgE-sensitization to Cor a 9 for correctly diagnosing hazelnut allergy, but this superiority was not statistically significant. Eller et al.[17] showed in a retrospective study that 94 % of challenge-positive children were sensitized to Cor a 9 or 14, but importantly the remaining 6 % (4 patients) only had mild symptoms at the food challenge (OAS, lip swelling or localized urticaria). This study concludes that sensitization to Cor a 14 is the best diagnostic marker, although not significantly superior to Cor a 9; results from the US also indicate the superiority of Cor a 14.[170] From the Netherlands, Masthoff et al. made a similar study to ours (however, this study also included adult patients); here, sensitization to Cor a 9 seemed to outperform sensitization to Cor a 14.[15] This study also showed that 13 % of the allergic children and a majority of the adult allergic patients (55 %) were not sensitized to Cor a 9 or 14, but in a majority of the adults, the diagnosis was based on patient history alone (no challenge). However, there will always be outliers or exceptions from the diagnostic "rules" and they might be more common than expected. Kirsten Beyer et al. showed that the IgE-ab level to Cor a 14 corresponding to a 90 % probability of challenge proven hazelnut allergy was as high as 47.8 kU_A/l (and for peanut allergy, the Ara h 2 level was 14.4 kU_A/l).[104]

So why are some patients with high-degree sensitization to Cor a 9 and Cor a 14 tolerant to hazelnuts? In our study, we observed a few hazelnut-tolerant patients with high IgE-ab levels to Cor a 9 or 14. Cross-reactivity might explain that some patients with high IgE-ab levels to Cor a 9 (but low Cor a 14) tolerated hazelnuts, as they had a verified peanut allergy with high IgE-ab titers to peanut and peanut components. A low allergen-specific IgE-abs/total IgE ratio might explain why some patients tolerate an allergen despite high IgE-levels to key components,[171] as we observed in one patient who had an IgE-abs to Cor a 14 IgE of 13.9 but a total serum IgE of > 5,000 kU/l.

6.1.1 CD-sens to hazelnut

All patients with a positive hazelnut challenge were positive also in CD-sens; the allergic patients having a median CD-sens of 8.9 (5.2–41). The hazelnut-tolerant patients' median level was substantially and significantly lower 0.05 (0.007–0.11) ($P < .0001$) and at the ROC-generated cut-off value of > 1.7, a 100 % sensitivity, 97 % specificity was reached for discrimination of

tolerance/subjective symptoms from objective symptoms at challenge. Of course, these results must be interpreted with great caution due to the small number of challenge-positive patients, especially when determining a cut-off value for clinical use. Elizur et al. analyzed BAT in children with allergies to one or more types of nuts (walnut, pecan, almond, cashew, pistachio, and hazelnut) and found that BAT was only superior to SPT when diagnosing hazelnut allergy; however, they used the CD-max method, not the CD-sens method.[172] In a study of German adults, CD-sens (CD63-upregulation after stimulation with hazelnut) was significantly higher in patients with oral symptoms (or mild urticaria) or systemic symptoms compared with sensitized patients with no symptoms at all. But CD-sens could not discriminate patients with mild symptoms from those with systemic reactions.[173]

In the patients with IgE-sensitization to Cor a 9/Cor a 14, CD-sens seems to be a valuable diagnostic tool as shown in the sensitivity analysis of CD-sens in this subgroup. Among hazelnut-tolerant patients with > 2 kU_A/l of IgE-abs to Cor a 9 or 14 (n = 8), the median CD-sens to hazelnut was very low, 0.035 (the highest observed value was 0.2), while the lowest CD-sens among allergic patients was 3.07. Thus, it seems like CD-sens can be used as a complementary test when the diagnosis remains uncertain after using CRD.

6.1.2 Strengths and limitations

We performed DBPCFC on all subjects and these challenges were performed by experienced staff. Blood samples were drawn at the same day as the first challenge. With the rather small study population, the low proportion (20 %) of subjects reacting at DBPCFC became the main limitation. These results do not indicate that 80 % of patients with a doctor's diagnosis of suspected hazelnut allergy are actually tolerant; our study population did not include patients who already had performed food challenges to hazelnut or patients with a history of moderate to severe anaphylaxis to hazelnut. It is also likely that the diagnostic accuracy in our study population (before DBPCFC) could have been improved by combining the results of SPT and analysis of IgE-abs to hazelnut and birch if these results were interpreted by a trained allergist.[41,87]

6.1.3 Clinical implications

In conclusion, both CRD and CD-sens facilitate hazelnut allergy diagnostics in children[15,17,90,170] but it is still not perfect (the situation among adults remains even more complex[15,173]). While the specificity with these new diagnostic tests can be increased, there is still a risk of over-diagnosing hazelnut allergy if we do not analyze all data carefully. A two-step procedure with history and IgE-testing including CRD, followed by CD-sens in selected cases, could further improve the diagnostic accuracy and decrease false positive rates and/or the need of food challenges.

6.2 PEANUT ORAL IMMUNOTHERAPY COMBINED WITH OMALIZUMAB

In the FASTX studies we have corroborated previous findings of omalizumab's potency in increasing peanut-allergic patients' tolerance level to peanut. We have also showed that peanut oral immunotherapy can induce desensitization to peanut, but treatment is long and often troublesome.

Omalizumab's ability to increase food-allergic patients' ability to tolerate the culprit foods has been proven in several studies.[36,37,39,40,138] In these studies, the drug has been dosed either based on patient weight and total IgE or all patients received the same dose. Irrespective of dosing method, some patients remain very sensitive to peanuts, e.g., in the study by MacGinnitie et al. 4/27 patients could not reach a 250 mg peanut protein dose during rapid up-dosing after omalizumab[39], and 1/9 patients

did not increase the tolerance to peanut at all, despite five months of omalizumab treatment in the study by Sampson et al.[37] This was not observed in the FASTX study where all patients could ingest >800 mg peanut protein and we believe that this is at least partially due to the fact that 15/23 received an elevated dose of omalizumab. A dose-response correlation has previously been described for anti-IgE-treatment,[138] but since we did not perform food challenges before the dose was increased in these 15 patients we cannot make a causal conclusion of the necessity to increase the dose in some patients.

Our results also provide strong support for the protective effects of omalizumab during oral immunotherapy. The up-dosing went smoothly and rapidly in most patients; all 23 patients reached the maintenance dose of 10 g peanut in a median time of ten weeks. Our results can be compared with the results from pOIT studies without omalizumab but with similar study populations to ours; Blumchen et al.[129] reported that 14/22 (64 %) patients reached a tolerated dose of 500 mg after seven months (median) of pOIT and four patients were excluded due to repeated allergic reactions, while Kukkonen et al.[174] found that 33/39 (85 %) were able to reach the maintenance dose (4 peanuts) in a median of about nine months. MacGinnitie et al. compared pOIT and omalizumab with pOIT and placebo[39] and they reported that there were fewer (but not significantly fewer) adverse events among omalizumab-treated patients; however, the omalizumab group consumed substantially higher doses of peanut. I am confident that if the pOIT doses had been matched, the difference in adverse events would have been more striking.[39] The major disadvantage of using omalizumab as adjunctive therapy to OIT is its high price, ~1,000 €/month. While there are no studies on cost-effectiveness for omalizumab on this indication (though there are studies for asthma[175]), it seems highly unlikely that long-term treatment protocols would prove to be cost-effective. I think it is safe to conclude that omalizumab facilitates OIT, at least for patients with a severe allergy. Whether it also improves long-term outcomes after the omalizumab treatment has been discontinued, for instance reducing AEs in OIT or achieving sustained unresponsiveness,[35] is doubtful.[36,39]

In total, 9/23 (39 %) of the patients in the our study met the primary end-point of tolerating pOIT at a dose of 2,800 mg of peanut for three months after discontinuation of omalizumab. Two more patients completed the protocol with a reduced pOIT (1,120/700 mg). We chose to include these two patients in the treatment success group for statistical analysis as the reason for dose-reduction was an abomination to the taste of peanuts, rendering an intention-to-treat success rate of 48 % (11/23). Per-protocol success rates are often reported in clinical trials; however, in studies like FASTX, I believe that outcomes should be reported as ITT. In per-protocol analysis, drop-outs or patients not adhering to protocol are excluded from analysis. When a treatment is associated with side effects or is tough in other aspects, patients will adhere worse to treatment and some will stop it completely. Taking FASTX as an example, the per-protocol success rate (65 %) was markedly higher than the ITT success rate (48 %); however, for at least 4/6 of the drop-outs, it was adverse events (including an abomination to the taste of peanuts) that made them decide to discontinue the treatment.

6.2.1 OIT vs. traditional AI (for allergies to pollen, pet, house dust mite etc.)

Sub-cutaneous immunotherapy (SCIT) for, e.g., pollen allergy, has been practiced for over a century and is by now a widely accepted treatment. SCIT and later also SLIT are now widely implemented for the treatment of several types of allergies. When I have talked about our research and OIT I have often been asked questions like “Why does SCIT work so well, while OIT does not?” or “Why are there so many allergic reactions in OIT?” First of all, traditional AI is not effective in all patients and adverse

events do occur. Secondly, traditional AI has been gradually refined and improved over the past century, whereas OIT has only been tried in small trials in the last 10–15 years.

AI mitigates allergies/allergic symptoms,[28] and reduces development of asthma and new IgE-sensitization in children.[176] However, it is rarely completely curative, evidence for long-term effects is scarce and efficacy differs from one allergen to another.[177] Severe adverse events are rare, but they do occur, with a frequency of adrenaline administration in the range of 0.1–0.2 % of SCIT doses[28,178] (comparable to the corresponding frequency in OIT[33,34]). However, since OIT is typically administered once daily, in contrast to roughly monthly injections in SCIT, the cumulative incidence of adverse events requiring epinephrine is higher in OIT. Table 6 makes a comparison of food OIT and SCIT for pollen allergy.

OIT	SCIT
Likely not curative, increased tolerance allows for more liberal approach to eating out, attending parties where nuts are served etc. ☺ Retained risk of having serious allergic reactions even after long-term OIT ☹	Most often not curative, reduction of symptoms can still be of value, milder symptoms, less medication ☺
Long treatment ☹	Long treatment ☹
Daily dosing. Avoid physical activity for two hours following OIT dose EVERY day ☹	~Once a month. ☺
Worse compliance, forgotten or postponed doses due to, e.g., infections might affect long-term efficacy ☹	Minor adjustments to dose intervals most likely have no effect on long-term effects ☺
High cumulative incidence of adverse events ☹	Low cumulative incidence of adverse events ☺
Adverse events occur at home ☹ Fewer visits to the clinic ☺	Adverse events occur at the clinic ☺ Monthly visits to the clinic ☹
No injections ☺	Injections ☹
No natural boosters, ingestion has to continue (?) to sustain the increased tolerance ☹	For pollen or house dust mite allergy and similar, natural boosters (exposure) might prolong positive effects ☺
Long-term efficacy? ☹	Long-term efficacy? ☹

Table 6. Comparison of OIT for food allergy and SCIT for pollen allergy.

6.2.2 Oral immunotherapy; does it work? Is it worth it?

Success rates for oral immunotherapy vary substantially from one study to another. Direct comparisons between studies are hampered by differences in study populations (age, severity of allergy), study protocols (duration, amount of peanut ingested, adjuvant treatments such as omalizumab,[39] or antihistamines[174]), and outcomes (target dose, intention-to-treat vs. per-protocol). These differences are important, since current evidence suggests that patients with a more severe allergy (at least in terms of sensitization levels) respond worse to treatment,[35,36,179] and that starting OIT at an early age seems to be beneficial.[70,119,180] The difference is obvious when primary outcomes differ from tolerating small amounts[129] to full servings.[35] Also, many OIT studies report results when OIT has been given for a rather short period of time (6–12 months), which might lead to an

underestimation of drop-out rates and hence an overestimation of the positive effects/tolerability of OIT.

Despite this, there is now solid evidence that oral immunotherapy, in most patients, can induce an increased tolerance[119] to several food allergens like egg,[130,181,182], milk,[36,181,183,184] and peanuts.[34,129,131,132,160,174,185] OIT has also been tried for other foods like wheat[186,187] and multiple foods.[188] Although we need more evidence, I can see no theoretical reason why OIT should be less effective for other IgE-mediated food allergies.

OIT can induce an increased tolerance, but it comes at a high price; patients going through OIT experience more systemic allergic reactions than those on avoidance diet.[189] However, our personal experience from this study and previous work by our group[23] and others[174] indicate that the patients think it is a price worth paying. How can more allergic reactions and daily ingestion of (almost unanimously disliked) peanuts be preferred over continuing with strict avoidance? I think there are several reasons for this: being able to tolerate small amounts of the allergen allows a patient to eat at restaurants, cafés and parties without asking questions and having to worry. Even though systemic allergic reactions occur, they are to some extent expected and I believe that most of us handle setbacks or difficulties in life better if they were expected.

6.3 OIT IN A NEAR FUTURE

6.3.1 Important questions in OIT

There are still many questions that need to be answered regarding OIT before it can be recommended as routine practice.

Who should we treat with OIT? Patients with severe allergies will likely benefit more from a successful treatment; on the other hand, these patients seem to have significantly poorer outcomes.[35,179] Severely allergic patients might benefit from omalizumab protection,[36] but will this be cost-effective?

For how long should OIT be given, and does it have to be administered daily? In studies evaluating sustained unresponsiveness after a couple of years of OIT, much of the increased tolerance was lost in a majority of patients after stopping OIT for weeks-months,[36,160,190] but it seems that SU becomes more likely when treatment is longer.[190] In the few published studies of long-term follow-up on OIT it appears that more liberal dosing schedules have been applied[130,191] and, as I interpret these reports, this did not affect outcome. There is currently at least one study comparing different dosing schedules in long-term peanut OIT.[192]

In which form should the food be ingested? Roasted peanuts, peanut flour and standardized (modified?) powder formulas (patented as a drug) have been tried. The cheapest, but least standardized, option would be to use peanuts straight from the grocery store, but one brand or type will differ from another when it comes to protein content, processing (roasting, degree of roasting) which will affect the allergenicity of the proteins. Boiled peanuts may decrease allergenicity while the ability of inducing protective immunological changes is maintained.[193]

6.3.2 Improving OIT

So far, OIT has shown potential as a disease-modifying treatment for food allergies, but we need to find methods to both increase the efficacy and decrease the number of adverse events before it can be implemented as routine practice at allergy clinics. Omalizumab has proven capable of reducing

adverse events in OIT, but it is an off-label treatment and hence it is unlikely that (the very high) costs will be covered by subsidies or health care insurances. One clinical implication is to seize the opportunity and start OIT in food-allergic patients who are treated with omalizumab for allergic asthma (but wait until the asthma is well-controlled).

Another appealing idea is to start OIT early in life. A secondary prevention for peanut allergy among infants at risk, recently performed within the LEAP study,[70] proved to be highly effective. Last year, very impressing results of pOIT in early childhood were reported by Vickery et al.[180] They showed a 78 % SU rate after a median of 2.5 years of OIT; additionally, no serious adverse events occurred, adrenaline was administered only once and the drop-out rate of 8 % was low in relation to the long treatment protocol.

A third option to increase the efficacy and safety of OIT would be to develop modified OIT preparations, where we could learn from the science of vaccine development. Effective vaccines induce a strong but selective immune response, production of protective antibodies in the absence of symptoms of the disease (or mild symptoms only). By recombinant technology, protein structures can be altered so that a hypoallergenic (reduced IgE-binding properties), but normo-immunogenic (or ideally, a hyper-immunogenic) molecule is created.[194] Conjugate extracts, e.g., with DNA sequences recognized by toll-like receptors[195,196] or protein sequences mimicking virus proteins,[197] are other appealing approaches that could be tried to improve OIT.

Finally, the taste of peanuts seems to be a major problem, at least for our study subjects. If it would be possible to process peanuts or produce a peanut imitation by, e.g., recombinant techniques, so as to alter the taste, I am confident that compliance would improve and drop-out rates would be lower.

7 ETHICAL CONSIDERATIONS

There are advantages and disadvantages to participation in medical studies. Table 7 lists potential advantages and disadvantages of participating in the FASTX study.

Advantages	Disadvantages
Potential cure. If partial effect, protection from accidental exposure to small amounts of peanut.	New treatment, will it work in our population? How long will the protection last? OIT-induced allergic reactions.
Omalizumab ameliorates other IgE-mediated diseases (e.g., asthma and allergy to pollen).	Painful injections. Risk of side effects from omalizumab. Blood samples drawn at several occasions.
Easy access to pediatric allergists and nurse in the study team. Omalizumab treatment for 1–2.5 years.	Time-consuming, 20–50 hospital visits. Daily peanut consumption; most patients detested the taste of peanuts. Cannot go on long trips > 4 weeks while on omalizumab.
	Peanut challenge prior to inclusion: potentially dangerous and causing the patients a lot of emotional stress.

Table 7. Advantages and disadvantages of taking part in the FASTX study.

As with all demanding treatments, the disease itself has to cause a significant burden in order to make the treatment worthwhile. When presenting the study to the potential study subjects, I was surprised at how positive they were towards participation. Their fear of ingesting peanuts by mistake was almost always present, which clearly affected their quality of life. When presented with the facts, such as the long study protocol, frequent visits, uncertainty regarding outcome and risk of allergic reactions (most certainly during food challenge prior to inclusion), the patients and their parents were almost unanimously positive. In fact, when inclusion criteria were not met, patients and parents were very disappointed. However, when treating children and adolescents, we always have to remember that the patient's wishes come first, not the parents'. Since parents of food-allergic children are often affected themselves, because of worries for their children, it is of utmost importance that we ascertain that the teenagers themselves want to take part in the study/therapy. I believe we tried to do this throughout the study. Most patients came to their visits alone, and if not, we tried to talk with them alone. During the long treatment period, some patients experienced difficulties with the therapy or life in general. During these times, we sometimes had a delicate task of encouraging our patients while not violating their voluntary study participation.

By increasing their tolerance to peanuts, we will probably also have increased the patients' quality of life. Patients with good or partial effect of OIT will also need fewer medical appointments, resulting in less need to be out of school/work. They will probably also need fewer drugs; these effects combined translate into economic benefits for the patient, families and society.

The major drawbacks of the FASTX study were not the frequent visits, blood samples, and shots of omalizumab. Rather, they were the long study period, unexpected allergic reactions, and long-term psychological stress that some patients experienced while ingesting peanuts. The major advantage is the hope of curing an otherwise (most likely) life-long disease. If successful, this would allow a patient to live a life with fewer restrictions: to eat anything, anywhere, without worrying and not having to carry around emergency treatment kits. For patients with problematic rhino-conjunctivitis or asthma, omalizumab offers transient relief while on therapy, and this therapy would not be given to them otherwise due to the very high costs.

Finally, in the bigger perspective, the results from the FASTX study and other studies on OIT or other types of AI for food allergy will help us to gradually improve the outcome of AI for food allergy by improving treatment protocols, selecting the right patients for treatment and finding strategies to minimize adverse events during therapy.

8 CONCLUSIONS

With the work performed in this thesis we believe that we have contributed to an improved situation for children and adolescents affected by an allergy to nuts or peanuts.

- Component-resolved diagnostics can improve the accuracy when diagnosing hazelnut allergy in pediatric patients.
- CD-sens to hazelnut may provide valuable additional information in cases when the diagnostic work-up using CRD has been inconclusive.
- The anti-IgE-ab omalizumab can induce a pronounced decreased sensitivity to peanuts which in turn allows for a safer practice of peanut oral immunotherapy in severely allergic patients.
- Peanut oral immunotherapy induces an increased tolerance to peanuts; the increased tolerance is at least partially explained by the production of protective allergen specific antibodies of IgG4-subtype.
- Despite the increased tolerance, allergic reactions continuously occur during pOIT. We need to find ways to minimize this major limitation before OIT can be widely implemented. Development of hypoallergenic OIT preparations, use of immunostimulatory adjuvants, and improved patient selection might help in accomplishing a safer and more effective treatment.

9 SAMMANFATTNING PÅ SVENSKA

Bakgrund: Förekomsten av allergi mot livsmedel har ökat under de senaste årtiondena och bland europeiska barn har närmare 8 % en allergi mot ett eller flera livsmedel. Men det finns också många barn som har fått en felaktig diagnos av matallergi på grund av brister i tillgängliga diagnostiska test, speciellt vid misstänkt allergi mot nötter eller jordnötter. Tack vare nya diagnostiska test, som komponent-diagnostik (CRD) och CD-sens har de diagnostiska möjligheterna förbättrats.

Den allvarligaste akuta reaktionen vid allergi, anafylaktisk reaktion, är bland svenska barn oftast orsakad av en allergi mot jordnötter eller nötter. För dessa svårt matallergiska patienter har det inte funnits någon tillgänglig behandling med möjlighet att förändra sjukdomsförloppet. Allergen immunterapi (hyposens/allergivaccination) har sedan länge erbjudits som behandling av patienter med uttalad pollenallergi eller allergi mot getingstick. Patienter med svåra matallergier har istället fått försöka undvika det de är allergiska mot och om olyckan skulle vara framme; använda de akutmediciner som dessa patienter ständigt bär med sig. Sedan 10-15 år har oral immunterapi (OIT) framkommit som en potentiell behandling för matallergier. Vid OIT äter patienten mycket små, men gradvis ökande doser av livsmedlet som framkallar allergisk reaktion varvid en ökad tolerans byggs upp. I de studier som är gjorda på OIT har allergiska reaktioner varit vanliga och patienter med en allvarligare allergi svarar sämre på behandlingen. OIT-metoden behöver förfinas innan den kan införas på bredare front som en behandling av matallergi. Läkemedlet omalizumab (anti-IgE-antikroppar) har visat sig kunna minska känsligheten för födoämnen bland allergiska patienter och kan underlätta OIT.

Mål: Hasselnötsstudien: Att utvärdera de nya diagnostiska testen CRD och CD-sens hos barn med en misstänkt hasselnötsallergi. FASTX-studien: Att utvärdera säkerhet och effekt av oral immunterapi med omalizumab som skyddande tilläggsbehandling bland allvarligt jordnötsallergiska ungdomar.

Metoder: I hasselnötsstudien mätte vi IgE-antikroppar mot hasselnötskomponenterna Cor a 1, Cor a 8, Cor a 9 och Cor a 14 hos 40 barn med en misstänkt hasselnötsallergi. Vi utvärderade också basofil allergenkänslighet (CD-sens) för hasselnöt och jämförde utfallen av dessa test med utfallen i hasselnötsprovokationer.
I FASTX-studien gavs omalizumab till 23 ungdomar med svår jordnötsallergi vilket syftade till att minska känsligheten mot jordnötter så att OIT kunde ges under säkrare former. Omalizumab titrerades vid behov upp till dess att CD-sens visade en utsläckt eller mycket låg reaktivitet mot jordnöt. Därefter genomfördes en jordnötsprovokation, där den minskade känsligheten mot jordnötter utvärderades. Jordnöts-OIT startades följande dag under fortsatt skydd av omalizumab. Efter att ha nått underhållsdosen på 10 g jordnötter så trappades den skyddande omalizumab-behandlingen successivt ned, med vägledning från CD-sens och den kliniska bilden.

Resultat: Hasselnötsprovokationerna visade att endast 8/40 av patienterna med en misstänkt hasselnötsallergi var allergiska mot hasselnötter. Träffsäkerheten hos de nya diagnostiska testen, CD-sens och komponentdiagnostik med IgE-antikroppar mot Cor a 9 och Cor 14, var överlägsen de tidigare tillgängliga testen (IgE-ab till hasselnöt, Cor a 1 och Cor a 8). Alla hasselnötsallergiska patienter hade IgE-ak mot Cor a 9 och Cor a 14.

CD-sens bland patienter allergiska mot hasselnöt var i median 8,9 jämfört med 0,05 hos toleranta patienter ($P = 0,05$).

Efter behandling med omalizumab klarade alla 23 patienter en jordnötsprovokation där de åt åtminstone 3 g jordnötter (median 10 g). Bland de 14 patienter som även genomgick en jordnötsprovokation före studiestart ökade den tolererade dosen 50 gånger (median). Dock krävde 15/23 patienter en ökad dos av omalizumab för att åstadkomma den hämning av CD-sens som krävdes för att de skulle få göra provokationen. Alla 23 patienter nådde framgångsrikt underhållsdosen på 10 g jordnötter. Efter 23 månader (median) med OIT hade 11/23 (48 %) av försökspersonerna kunnat avsluta omalizumab-behandlingen medan de fortsatte OIT och kunde därefter framgångsrikt genomgå en ny jordnötsprovokation. Systemiska allergiska reaktioner förekom med en frekvens av 0,3 % av OIT-doserna (n = 43) och adrenalin gavs efter 0,1 % av doserna. De patienter som var framgångsrikt behandlade hade signifikant lägre CD-sens och lägre nivåer av IgE-ak mot jordnöt- och jordnötskomponenterna Ara h 1, Ara h 2 och Ara h 3 vid studiestart jämfört med patienter som inte kunde avbryta den skyddande omalizumab-behandlingen. OIT gav en ökning av skyddande IgG4-ak mot jordnöt, Ara h2 och Ara h 6 som var signifikant högre hos framgångsrikt behandlade patienter. Sex av de 23 patienterna hoppade av studien, främst på grund av rädsla för allergiska reaktioner och en avsky för smaken av jordnötter.

Slutsatser: CD-sens mot hasselnöt och komponentdiagnostik kan förbättra träffsäkerheten vid diagnostik av hasselnötsallergi hos barn.

Omalizumab-behandling ger en uttalat sänkt känslighet för jordnötter hos ungdomar med svår jordnötsallergi vilket i sin tur ger förutsättningar för att genomföra OIT under säkrare former. Den minskade känsligheten förklaras åtminstone delvis av produktionen av skyddande allergenspecifika antikroppar av IgG4-subtyp.

Trots den ökade toleransen uppstår allergiska reaktioner kontinuerligt under OIT. Innan OIT kan implementeras i större utsträckning måste vi hitta sätt att minimera behandlingsrelaterade allergiska reaktioner. Hypoallergena OIT-preparat, användning av immunstimulerande tilläggsbehandlingar och att hitta sätt att välja ut patienter med högre sannolikhet för positivt behandlingssvar kan vara sätt att åstadkomma detta.

10 ACKNOWLEDGEMENTS

There are so many people who have helped me in one way or another throughout my doctoral studies. I have probably forgotten to mention a few who really should have been mentioned, and am sorry if that is the case.

When conducting clinical research, you can achieve nothing without patients. So, first of all, I would like to thank **all the patients**, and their parents, who participated in the Hazelnut and FASTX studies, for their time and effort. For the 23 brave adolescents taking part in the FASTX study: It has been a pleasure being one of your physicians throughout the study period. Besides the fact that you have all been so kind and polite at all times, even when the treatment we put you through felt tough or did not work as well as we had hoped, you have also provided us with so much valuable information on OIT and omalizumab treatment. Also, as a pediatrician, I have learned so much from you that will make me a better physician when it comes to taking care of adolescents. I wish you all the best!

My supervisors, **Caroline Nilsson, Anna Nopp, Gunnar Lilja** and **Mirja Vetander**; what a team! You have complemented each other very well as supervisors. **Caroline**, you are one brave, hardworking and busy doctor and researcher: full of ideas and profound knowledge in the field of (food) allergy. As a supervisor, you have always given me the time, support and help I needed, but you have also been responsive to my thoughts and ideas and allowed me to make important decisions and work independently. **Anna**, thank you for teaching me everything I know about basophils and most of what I know about laboratory work. When I've asked for your help, usually in a late night e-mail, I've always found your response in my inbox early the following morning. As the "non-physician supervisor" you have many times provided me with a different view on how to do things, or how to report things, which I believe improved our work and our scientific reports. **Gunnar,** just like Anna, you always helped me out within hours of my asking for your help. Calm, knowing, encouraging and funny – you are always nice to be around, at the clinic, lunch table or research meetings. **Mirja**, you have been as much a co-worker as a supervisor, and I couldn't have asked for anyone better suited for either of those two roles. For all future PhD students, here is a piece of advice: enroll a "junior supervisor" like Mirja. It has been so great to seek (and get) support from someone who really remembers what it is like during the tough periods as a PhD student.

Ann-Charlotte Sundqvist. First of all, you are a great nurse for our allergy patients; but in particular you are great with the adolescents, they really confide in you. Secondly, without you I wouldn't be standing here today: you have put a tremendous amount of work into the FASTX and Hazelnut studies: finding patients, making appointments, giving thousands of omalizumab injections, arranging blood sample transports etc. Thank you!

Gunnar Johansson and **Magnus Borres**: From Uppsala, with expert knowledge on allergy and allergy diagnostics, and also my co-authors. I've enjoyed being around you, not only because I've learned a lot during those occasions, but also because you are both funny and kind.

Stefan Johansson, my external mentor. Thank you for nice talks about my research, research in general, and future career choices.

Eva Berggren-Broström, head of Sachs' Children and Youth Hospital, thank you for giving me the opportunity to conduct my research at the clinic and for allowing a flexible schedule for my research vs. clinical duties. Also, thank you for positive and encouraging words along the way.

Bodil Schiller, my former boss, and **Fredrik Stenius,** my current boss. You have both been very supportive of my research and allowed me to plan and conduct my clinical and research work as I have wished, whenever possible.

Anna-Karin Edstedt Bonamy, thank you for all your advice and encouragement along the way and for convincing me to apply to the research school in epidemiology. I really didn't think I had a shot at it, but you made me believe it was worth a try.

Carina Wallén Thank you for all your work with analyses of IgE and IgG antibodies.

Zekiye Cansu and **Frida Kalm** for all the CD-sens analyses and an extra thank you to Zekiye for the time I spent with you at the basophil lab when you taught me how to perform CD-sens.

Eva Sverremark Ekström and **Claudia Carvalho Queiroz** at the immunology unit at Stockholm University for taking care of lymphocyte samples within the FASTX study. I would also like to thank **Eva** and **Anna-Lena Spetz** for a great immunology course at Stockholm University.

All the staff at Sachs' allergy unit and research center. **Natalia Ballardini** and **Erik Melén** in particular for your encouragement, advice and good discussions, **Susanne Glaumann** for your much needed help with graphs when deadlines were approaching and for good discussions and **Agneta Janson Roth** for preparing chocolate balls for the blinded hazelnut challenges.

Staffan Arvidsson, thank you for all your work with the FASTX database.

Gabriella Bröms, director of studies at the KI research school in epidemiology: Thank you for organizing the best set of courses I've ever taken and for your excellent lectures.

My current and previous roommates at Sachs': We talked when we felt like talking, we worked when we had to work, it has been a pleasure being your roommate! **Susanna Klevebro**, **Björn Lundberg**, **Lina Schollin Ask,** in particular, for nice talks and sharing of thoughts about being a doctoral student, resident physician, and parent, and on how to combine these; now and in our future careers as pediatricians and researchers(?).

Sanam Zoghi Scott, I've known you since medical school and you are my best friend at Sachs'. You make Sachs' (and this world) a happier place.

Anette Norberg, **Rosie Andersson, Caroline Fransson** and **Anita Järvinen**, medical secretaries at Sachs', and **Jeanette Öhrman** at KI SÖS: thank you for your patience when helping me out with all the paper forms and other practicalities throughout my doctoral studies.

Hans Linder, my clinical supervisor as a resident physician; we might not have had quite as many supervisor-student sessions as *Socialstyrelsen* would have liked, but why make things complicated? I've always felt that I've had your support, backing me up when needed.

My informal external mentor **Björn Pasternak**; clinical epidemiologist at KI, but most importantly: a very dear friend. Thank you for the thousands of hours we've spent at golf courses, tennis courts, travelling and with our families, when we have had very fruitful (at least for me) discussions on medical research (in addition to tons of meaningless BS).

To my **friends** from Gotland, Stockholm, and Uppsala, or wherever you are from: I treasure our friendships and I hope that **you** will have the time for dinners, sports, and coffee now that I finally do.

To my father-in-law, **Tadeusz Zajączkowski**: thank you for your kindness and the great hospitality you show us when we visit you in Gdańsk. To my mother-in-law, **Kristina Roszek**: thank you for all the help and support that you and **Leszek** have given to my family, especially during these last busy years.

My parents, **Margaretha Blomgren** and **Hans Brandström**: thank you for raising me and my siblings in a loving home. You have always been very supportive. I am grateful that you made me push my limits at times when I thought it was okay to settle for "good enough." To my brothers and sisters, **Gustav, Maja, Samuel** and **Anna**: you are smart, kind, funny, and generous, you have all been very supportive and it is just a pleasure to hang out with you. **Frida, Bertil, Arne, Anna O** and **Klas**: I'm so happy that we're now family; family reunions are even better with you!

Monika, the love of my life and my beautiful wife; you are simply the best! Thank you for being who you are and for being the perfect mother to our children. **Emil** och **Julia**, ni är det bästa som finns, jag älskar er! Nu när boken är klar skall vi leka mycket, mycket mer!

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